WO05067460

Publication Title:

No title available

Abstract:

Abstract not available for WO05067460 Data supplied from the esp@cenet database - Worldwide

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(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2004/034693

(22) International Filing Date: 15 October 2004 (15.10.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/532,696 24 December 2003 (24.12.2003) US
60/602,588 18 August 2004 (18.08.2004) US
60/615,548 1 October 2004 (01.10.2004) US
60/617,564 7 October 2004 (07.10.2004) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: EPHA2 VACCINES

(57) Abstract: The present invention relates to methods and compositions designed for the treatment, management, or prevention of cancer, particularly metastatic cancer, and hyperproliferative diseases involving EphA2-expressing cells. In one embodiment, the methods of the invention comprise the administration of an effective amount of an EphA2 antigenic peptide to elicit an immune response against the EphA2-expressing cells. In other embodiments, the methods of the invention entail the use of an EphA2 expression vehicle, such as a naked nucleic acid or viral vector. In yet other embodiments, the methods of the invention comprise the use of adoptive immunotherapy with autologous or non-autologous antigen presenting cells that are sensitized with one or more EphA2 antigenic peptides. The invention also provides pharmaceutical compositions comprising one or more EphA2 antigenic peptides, expression vehicles or antigen-presenting cells of the invention either alone or in combination with one or more other agents useful for cancer therapy.

EphA2 VACCINES

This application is entitled to and claims priority benefit to U.S. provisional application Serial No. 60/532,696, filed December 24, 2003, U.S. provisional application Serial No. 60/602,588, filed August 18, 2004, U.S. provisional application Serial No. _____ , filed October 1, 2004 (Attorney Docket No. 10271-143-888), and

5   U.S. provisional application Serial No. _____ , filed October 7, 2004 (Attorney Docket No. 10271-148-888), each of which is incorporated herein by reference in its entirety.

1.   FIELD OF THE INVENTION

[0001]   The present invention relates to methods and compositions designed for the

10   treatment, management, or prevention of hyperproliferative cell disease. The present invention further relates to methods and compositions for eliciting an immune response against hyperproliferative cells. The methods of the invention comprise the administration of an effective amount of an EphA2 vaccine, comprising, for example, EphA2 antigenic peptides or an EphA2 antigenic peptide expression vehicle. The invention also provides

15   pharmaceutical compositions comprising one or more EphA2 antigenic peptides or peptide expression vehicles of the invention either alone or in combination with one or more other agents useful for therapy of hyperproliferative cell disorders.

2.   BACKGROUND OF THE INVENTION

2.1   Hyperproliferative Diseases

20   #### 2.1.1   Cancer

[0002]   A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and

25   spread to distant sites to cause death (for review, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Cancer can arise in many sites of the body and behaves differently depending upon its origin. Cancerous cells destroy the part of the body in which they originate and then spread to other part(s) of the body where they start new growth and cause more destruction.

[0003]   More than 1.2 million Americans develop cancer each year. Cancer is the second leading cause of death in the United States and, if current trends continue, cancer is expected to be the leading cause of death by the year 2010. Lung and prostate cancer are the top cancer killers for men in the United States. Lung and breast cancer are the top cancer killers for women in the United States. One in two men in the United States will be diagnosed with cancer at some time during his lifetime. One in three women in the United States will be diagnosed with cancer at some time during her lifetime.

[0004]   A cure for cancer has yet to be found. Current treatment options, such as surgery, chemotherapy and radiation treatment, are often either ineffective or present serious side effects.

2.1.2 Metastasis

[0005]   The most life-threatening forms of cancer often arise when a population of tumor cells gains the ability to colonize distant and foreign sites in the body. These metastatic cells survive by overriding restrictions that normally constrain cell colonization into dissimilar tissues. For example, typical mammary epithelial cells will generally not grow or survive if transplanted to the lung, yet lung metastases are a major cause of breast cancer morbidity and mortality. Recent evidence suggests that dissemination of metastatic cells through the body can occur long before clinical presentation of the primary tumor. These micrometastatic cells may remain dormant for many months or years following the detection and removal of the primary tumor. Thus, a better understanding of the mechanisms that allow for the growth and survival of metastatic cells in a foreign microenvironment is critical for the improvement of therapeutics designed to fight metastatic cancer and diagnostics for the early detection and localization of metastases.

2.1.3 Cancer Cell Signaling

[0006]   Cancer is a disease of aberrant signal transduction. Aberrant cell signaling overrides anchorage-dependent constraints on cell growth and survival (Rhim *et al.*, 1997, *Crit. Rev. in Oncogenesis* 8:305; Patarca, 1996, *Crit. Rev. in Oncogenesis* 7:343; Malik *et al.*, 1996, *Biochimica et Biophysica Acta* 1287:73; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105). Tyrosine kinase activity is induced by extracellular matrix (ECM) anchorage and indeed, the expression or function of tyrosine kinases is usually increased in malignant cells (Rhim *et al.*, 1997, *Critical Reviews in Oncogenesis* 8:305; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105; Hunter, 1997, *Cell* 88:333). Based on evidence that tyrosine kinase activity is necessary for malignant cell growth, tyrosine kinases have been targeted with new therapeutics (Levitzki *et al.*, 1995, *Science* 267:1782; Kondapaka *et al.*, 1996, *Mol. & Cell. Endocrinol.* 117:53; Fry *et al.*, 1995, *Curr. Opin. in BioTechnology*

6:662). Unfortunately, obstacles associated with specific targeting to tumor cells often limit the application of these drugs. In particular, tyrosine kinase activity is often vital for the function and survival of benign tissues (Levitzki *et al.*, 1995, *Science* 267:1782). To minimize collateral toxicity, it is critical to first identify and then target tyrosine kinases that
5 are selectively overexpressed in tumor cells.

2.1.4 Cancer Therapy

[0007]   Barriers to the development of anti-metastasis agents have been the assay systems that are used to design and evaluate these drugs. Most conventional cancer therapies target rapidly growing cells. However, cancer cells do not necessarily grow more
10 rapidly but instead survive and grow under conditions that are non-permissive to normal cells (Lawrence and Steeg, 1996, *World J. Urol.* 14:124-130). These fundamental differences between the behavior of normal and malignant cells provide opportunities for therapeutic targeting. The paradigm that micrometastatic tumors have already disseminated throughout the body emphasizes the need to evaluate potential chemotherapeutic drugs in
15 the context of a foreign and three-dimensional microenvironment. Many standard cancer drug assays measure tumor cell growth or survival under typical cell culture conditions (*i.e.*, monolayer growth). However, cell behavior in two-dimensional assays often does not reliably predict tumor cell behavior *in vivo*.

[0008]   Currently, cancer therapy may involve surgery, chemotherapy, hormonal
20 therapy and/or radiation treatment to eradicate neoplastic cells in a patient (*see, e.g.*, Stockdale, 1998, "Principles of Cancer Patient Management," in Scientific American: Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. IV). Recently, cancer therapy may also involve biological therapy or immunotherapy. All of these approaches can pose significant drawbacks for the patient. Surgery, for example, may be
25 contraindicated due to the health of the patient or may be unacceptable to the patient. Additionally, surgery may not completely remove the neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue, and radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent and, although it can be effective, is often used to
30 prevent or delay recurrence of cancer after other treatments have removed the majority of the cancer cells. Biological therapies/immunotherapies are limited in number and each therapy is generally effective for only a very specific type of cancer.

[0009]   With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A significant majority of cancer chemotherapeutics act by
35 inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of the

deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division (see, e.g., Gilman et al., 1990, Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed. (Pergamom Press, New York)). These agents, which include alkylating agents, such as nitrosourea, anti-metabolites, such as methotrexate and

5   hydroxyurea, and other agents, such as etoposides, campathecins, bleomycin, doxorubicin, daunorubicin, etc., although not necessarily cell cycle specific, kill cells during S phase because of their effect on DNA replication. Other agents, specifically colchicine and the vinca alkaloids, such as vinblastine and vincristine, interfere with microtubule assembly resulting in mitotic arrest. Chemotherapy protocols generally involve administration of a

10 combination of chemotherapeutic agents to increase the efficacy of treatment.

[0010]   Despite the availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks (see, e.g., Stockdale, 1998, "Principles Of Cancer Patient Management" in Scientific American Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. X). Almost all chemotherapeutic agents are toxic, and chemotherapy

15 causes significant, and often dangerous, side effects, including severe nausea, bone marrow depression, immunosuppression, etc. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other

20 drugs, even those agents that act by mechanisms different from the mechanisms of action of the drugs used in the specific treatment; this phenomenon is termed pleiotropic drug or multidrug resistance. Thus, because of drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

[0011]   There is a significant need for alternative cancer treatments, particularly for

25 treatment of cancer that has proved refractory to standard cancer treatments, such as surgery, radiation therapy, chemotherapy, and hormonal therapy. Further, it is uncommon for cancer to be treated by only one method. Thus, there is a need for development of new therapeutic agents for the treatment of cancer and new, more effective, therapy combinations for the treatment of cancer.

30   **2.1.5   Other Hyperproliferative Disorders**

          2.1.5.1   Asthma

[0012]   Asthma is a disorder characterized by intermittent airway obstruction. In western countries, it affects 15% of the pediatric population and 7.5% of the adult population (Strachan et al., 1994, *Arch. Dis. Child* 70:174-178). Most asthma in children

and young adults is initiated by IgE mediated allergy (atopy) to inhaled allergens such as house dust mite and cat dander allergens. However, not all asthmatics are atopic, and most atopic individuals do not have asthma. Thus, factors in addition to atopy are necessary to induce the disorder (Fraser *et al.*, eds.,1994, Synopsis of Diseases of the Chest: 635-53

5   (WB Saunders Company, Philadelphia); Djukanovic *et al.*, 1990, *Am. Rev. Respir. Dis.* 142:434-457). Asthma is strongly familial, and is due to the interaction between genetic and environmental factors. The genetic factors are thought to be variants of normal genes ("polymorphisms") which alter their function to predispose to asthma.

[0013]   Asthma may be identified by recurrent wheeze and intermittent air flow

10   limitation. An asthmatic tendency may be quantified by the measurement of bronchial hyper-responsiveness in which an individual's dose-response curve to a broncho-constrictor such as histamine or methacholine is constructed. The curve is commonly summarized by the dose which results in a 20% fall in air flow (PD20) or the slope of the curve between the initial air flow measurement and the last dose given (slope).

15   [0014]   In the atopic response, IgE is produced by B-cells in response to allergen stimulation. These antibodies coat mast cells by binding to the high affinity receptor for IgE and initiate a series of cellular events leading to the destabilization of the cell membrane and release of inflammatory mediators. This results in mucosal inflammation, wheezing, coughing, sneezing and nasal blockage.

20   [0015]   Atopy can be diagnosed by (i) a positive skin prick test in response to a common allergen; (ii) detecting the presence of specific serum IgE for allergen; or (iii) by detecting elevation of total serum IgE.

2.5.1.2 COPD

[0016]   Chronic obstructive pulmonary disease (COPD) is an umbrella term

25   frequently used to describe two conditions of fixed airways disorders, chronic bronchitis and emphysema. Chronic bronchitis and emphysema are most commonly caused by smoking; approximately 90% of patients with COPD are or were smokers. Although approximately 50% of smokers develop chronic bronchitis, only 15% of smokers develop disabling airflow obstruction. Certain animals, particularly horses, suffer from COPD as

30   well.

[0017]   The airflow obstruction associated with COPD is progressive, may be accompanied by airway hyperactivity, and may be partially reversible. Non-specific airway hyper-responsiveness may also play a role in the development of COPD and may be predictive of an accelerated rate of decline in lung function.

[0018]   COPD is a significant cause of death and disability. It is currently the fourth

leading cause of death in the United States and Europe. Treatment guidelines advocate

early detection and implementation of smoking cessation programs to help reduce morbidity

and mortality due to the disorder. However, early detection and diagnosis has been difficult

5   for a number of reasons. COPD takes years to develop and acute episodes of bronchitis

often are not recognized by the general practitioner as early signs of COPD. Many patients

exhibit features of more than one disorder (*e.g.*, chronic bronchitis or asthmatic bronchitis)

making precise diagnosis a challenge, particularly early in the etiology of the disorder.

Also, many patients do not seek medical help until they are experiencing more severe

10   symptoms associated with reduced lung function, such as dyspnea, persistent cough, and

sputum production. As a consequence, the vast majority of patients are not diagnosed or

treated until they are in a more advanced stage of the disorder.

2.1.5.3   Mucin

[0019]   Mucins are a family of glycoproteins secreted by the epithelial cells

15   including those at the respiratory, gastrointestinal and female reproductive tracts. Mucins

are responsible for the viscoelastic properties of mucus (Thornton *et al.*, 1997, *J. Biol.*

Chem. 272:9561-9566). Nine mucin genes are known to be expressed in man: MUC 1,

MUC 2, MUC 3, MUC 4, MUC 5AC, MUC 5B, MUC 6, MUC 7 and MUC 8 (Bobek *et al.*,

1993, *J. Biol. Chem.* 268:20563-9; Dusseyn *et al.*, 1997, *J. Biol. Chem.* 272:3168-78;

20   Gendler *et al.*, 1991, *Am. Rev.Resp. Dis.* 144:S42-S47; Gum *et al.*, 1989, *J. Biol. Chem.*

264:6480-6487; Gum *et al.*, 1990, *Biochem. Biophys. Res. Comm.* 171:407-415; Lesuffleur

et al., 1995, J. *Biol. Chem.* 270:13665-13673; Meerzaman *et al.*, 1994, *J. Biol. Chem.*

269:12932-12939; Porchet *et al.*, 1991, *Biochem. Biophys. Res. Comm.* 175:414-422;

Shankar *et al.*, 1994, *Biochem. J.* 300:295-298; Toribara *et al.*, 1997, J. *Biol. Chem.*

25   272:16398-403). Many airway disorders such chronic bronchitis, chronic obstructive

pulmonary disease, bronchietactis, asthma, cystic fibrosis and bacterial infections are

characterized by mucin overproduction (Prescott *et al.*, *Eur. Respir. J.*, 1995, 8:1333-1338;

Kim *et al.*, *Eur. Respir. J.*, 1997, 10:1438; Steiger *et al.*, 1995, *Am. J. Respir. Cell Mol.*

Biol., 12:307-314). Mucociliary impairment caused by mucin hypersecretion leads to

30   airway mucus plugging which promotes chronic infection, airflow obstruction and

sometimes death. For example, COPD, a disorder characterized by slowly progressive and

irreversible airflow limitation, is a major cause of death in developed countries. The

respiratory degradation consists mainly of decreased luminal diameters due to airway wall

thickening and increased mucus caused by goblet cell hyperplasia and hypersecretion.

35   Epidermal growth factor (EGF) is known to upregulate epithelial cell proliferation, and

mucin production/secretion (Takeyama *et al.*, 1999, *Proc. Natl. Acad. Sci. USA* 96:3081-6;

Burgel *et al.*, 2001, *J. Immunol.* 167:5948-54). EGF also causes mucin-secreting cells, such

as goblet cells, to proliferate and increase mucin production in airway epithelia (Lee *et al.*,

2000, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 278:185-92; Takeyama *et al.*, 2001, *Am. J.*

5   *Respir. Crit. Care. Med.* 163:511-6; Burgel *et al.*, 2000, *J. Allergy Clin. Immunol.* 106:705-

12). Historically, mucus hypersecretion has been treated in two ways: physical methods to

increase clearance and mucolytic agents. Neither approach has yielded significant benefit

to the patient or reduced mucus obstruction. Therefore, it would be desirable to have

methods for reducing mucin production and treating the disorders associated with mucin

10 hypersecretion.

2.1.5.4   Restenosis

[0020]   Vascular interventions, including angioplasty, stenting, atherectomy and

grafting are often complicated by undesirable effects. Exposure to a medical device which

is implanted or inserted into the body of a patient can cause the body tissue to exhibit

15 adverse physiological reactions. For instance, the insertion or implantation of certain

catheters or stents can lead to the formation of emboli or clots in blood vessels. Other

adverse reactions to vascular intervention include endothelial cell proliferation which can

lead to hyperplasia, restenosis, *i.e.* the re-occlusion of the artery, occlusion of blood vessels,

platelet aggregation, and calcification. Treatment of restenosis often involves a second

20 angioplasty or bypass surgery. In particular, restenosis may be due to endothelial cell injury

caused by the vascular intervention in treating a restenosis.

[0021]   Angioplasty involves insertion of a balloon catheter into an artery at the site

of a partially obstructive atherosclerotic lesion. Inflation of the balloon is intended to

rupture the intima and dilate the obstruction. About 20 to 30% of obstructions reocclude in

25 just a few days or weeks (Eltchaninoff *et al.*, 1998, *J. Am Coll. Cardiol.* 32: 980-984). Use

of stents reduces the re-occlusion rate, however a significant percentage continues to result

in restenosis. The rate of restenosis after angioplasty is dependent upon a number of factors

including the length of the plaque. Stenosis rates vary from 10% to 35% depending the risk

factors present. Further, repeat angiography one year later reveals an apparently normal

30 lumen in only about 30% of vessels having undergone the procedure.

[0022]   Restenosis is caused by an accumulation of extracellular matrix containing

collagen and proteoglycans in association with smooth muscle cells which is found in both

the atheroma and the arterial hyperplastic lesion after balloon injury or clinical angioplasty.

Some of the delay in luminal narrowing with respect to smooth muscle cell proliferation

may result from the continuing elaboration of matrix materials by neointimal smooth

muscle cells. Various mediators may alter matrix synthesis by smooth muscle cells *in vivo*.

2.1.5.5 Neointimal Hyperplasia

[0023]   Neointimal hyperplasia is the pathological process that underlies graft

5   atherosclerosis, stenosis, and the majority of vascular graft occlusion. Neointimal

hyperplasia is commonly seen after various forms of vascular injury and a major component

of the vein graft's response to harvest and surgical implantation into high-pressure arterial

circulation.

[0024]   Smooth muscle cells in the middle layer (*i.e.* media layer) of the vessel wall

10   become activated, divide, proliferate and migrate into the inner layer (*i.e.* intima layer). The

resulting abnormal neointimal cells express pro-inflammatory molecules, including

cytokines, chemokines and adhesion molecules that further trigger a cascade of events that

lead to occlusive neointimal disease and eventually graft failure.

[0025]   The proliferation of smooth muscle cells is a critical event in the neointimal

15   hyperplastic response. Using a variety of approaches, studies have clearly demonstrated

that blockade of smooth muscle cell proliferation resulted in preservation of normal vessel

phenotype and function, causing the reduction of neointimal hyperplasia and graft failure.

[0026]   Existing treatments for the indications discussed above is inadequate; thus,

there exists a need for improved treatments for the above indications.

20   **2.2   EphA2**

[0027]   EphA2 is a 130 kDa receptor tyrosine kinase that is expressed in adult

epithelia, where it is found at low levels and is enriched within sites of cell-cell adhesion

(Zantek *et al*, 1999, *Cell Growth & Differentiation* 10:629; Lindberg *et al.*, 1990, *Molecular*

& Cellular Biology 10:6316). This subcellular localization is important because EphA2

25   binds ligands (known as EphrinsA1 to A5) that are anchored to the cell membrane (Eph

Nomenclature Committee, 1997, *Cell* 90:403; Gale *et al.*, 1997, *Cell & Tissue Research*

290: 227). The primary consequence of ligand binding is EphA2 autophosphorylation

(Lindberg *et al.*, 1990, *supra*). However, unlike other receptor tyrosine kinases, EphA2

retains enzymatic activity in the absence of ligand binding or phosphotyrosine content

30   (Zantek *et al.*, 1999, *supra*). EphA2 is upregulated on a large number hyperproliferating

cells, including aggressive carcinoma cells.

3.   SUMMARY OF THE INVENTION

[0028]   EphA2 is overexpressed and functionally altered in a large number of

malignant carcinomas. EphA2 is an oncoprotein and is sufficient to confer metastatic

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potential to cancer cells. EphA2 is also associated with other hyperproliferating cells and is implicated in diseases caused by cell hyperproliferation. The present invention stems from the inventors' discovery that administration of an expression vehicle for an EphA2 antigenic peptide to a subject provides beneficial therapeutic and prophylactic benefits against hyperproliferative cell disorders involving EphA2 overexpressing cells. Without being bound by any mechanism or theory, it is believed that the therapeutic and prophylactic benefit is the result of an immune response elicited against the EphA2 antigenic peptide.

[0029]   The present invention thus provides EphA2 vaccines and methods for their use. The EphA2 vaccines of the present invention can elicit or mediate a cellular immune response, a humoral immune response, or both. Where the immune response is a cellular immune response, it can be a Tc, Th1 or a Th2 immune response. In a specific embodiment, the immune response is a Th2 cellular immune response. In specific embodiments, the immune response is a CD8 response and/or a CD4 response.

[0030]   In a preferred embodiment, an EphA2 vaccine of the invention comprises or encodes one or more epitopes on EphA2 that is selectively exposed or increased on cancer cells relative to not non-cancer cells (i.e., normal, healthy cells or cells that are not hyperproliferative). In one embodiment, the cancer is of an epithelial cell origin. In other embodiments, the cancer is a cancer of the skin, lung, colon, prostate, breast, ovary, eosophageal, bladder, or pancreas or is a renal cell carcinoma or a melanoma. In another embodiment, the cancer is of a T cell origin. In yet other embodiments, the cancer is a leukemia or a lymphoma.

[0031]   In a preferred embodiment, the methods and compositions of the invention are used to prevent, treat or manage EphA2-expressing tumor metastases. In a preferred embodiment, the EphA2-expressing cells against which an immune response is sought ("target cells") overexpress EphA2 relative to a normal healthy cell of the same type as assessed by an assay described herein or known to one of skill in the art (e.g., an immunoassay such as an ELISA or a Western blot, a Northern blot or RT-PCR). In a preferred embodiment, less EphA2 on the target cells is bound to ligand compared to a normal, healthy cell of the same type, either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in amount of EphA2 relative to ligand. In another embodiment, approximately 10% or less, approximately 15% or less, approximately 20% or less, approximately 25% or less, approximately 30% or less, approximately 35% or less, approximately 40% or less, approximately 45% or less, approximately 50% or less, approximately 55% or less, approximately 60% or less, approximately 65% or less,

approximately 70% or less, approximately 75% or less, approximately 80% or less, approximately 85% or less, approximately 90% or less, or approximately 95% or less of EphA2 on the target cells is bound to ligand (e.g., EphrinA1) compared to a normal, healthy cell of the same type as assessed by an assay known in the art (e.g., an immunoassay). In another embodiment, 1-10 fold, 1-8 fold, 1-5 fold, 1-4 fold or 1-2 fold, or 1 fold, 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, or 10 fold less EphA2 on target cells is bound to ligand (e.g., EphrinA1) compared to a normal, healthy cell of the same type as assessed by an assay known in the art (e.g., an immunoassay).

[0032]   The present invention provides methods of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual an EphA2 vaccine in an amount effective to elicit an immune response against an EphA2-expressing cell. The present invention also provides a method of treating, preventing, or managing a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to an individual an EphA2 vaccine in an amount effective treat, manage or prevent the hyperproliferative disorder (e.g., a neoplastic hyperproliferative disorder and a non-neoplastic hyperproliferative disorder). The present invention further provides EphA2 vaccines useful for eliciting an immune response against an EphA2-expressing cell and/or for treating, preventing or managing a hyperproliferative disorder of EphA2-expressing cells.

[0033]   The EphA2 vaccines may comprise an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, an antigen presenting cell sensitized with an EphA2 antigenic peptide, or an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody.

[0034]   In embodiments where an EphA2 vaccine comprises an EphA2 antigenic peptide, the vaccine may further comprise an adjuvant, or a heat shock protein bound to the EphA2 antigenic peptide.

[0035]   In certain embodiments, the EphA2 antigenic peptide comprises a protein transduction domain, for example the protein transduction domain is the Antennapedia or the HIV tat protein transduction domain.

[0036]   In certain embodiments in which an EphA2 vaccine comprises an EphA2 antigenic peptide expression vehicle, the expression vehicle can be a nucleic acid, preferably DNA, encoding said EphA2 antigenic peptide operably linked to a promoter. The DNA can be conjugated to a carrier, including but not limited to an asialoglycoprotein carrier, a transferrin carrier, or a polymeric IgA carriers.

[0037]   In other embodiments, the expression vehicle is an infectious agent comprising a nucleic acid, said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic peptide operably linked to a promoter. Preferably, the sequence encoding said EphA2 antigenic peptide is codon-optimized for expression in said infectious agent

5   and/or the infectious agent is coated with a reagent that targets the infectious agent to EphA2-expressing cells (such as an EphA2 antibody) or to antigen-presenting cells.

[0038]   In a specific embodiment, the infectious agent for use as an EphA2 expression vehicle in accordance with the methods and compositions of the invention is a bacterium. Preferred bacteria for administration to human subjects are attenuated, for

10   example, are attenuated in their tissue tropism or ability to spread from cell to cell. In a preferred embodiment, the bacteria are engineered to express an EphA2 antigenic peptide that is secreted from the bacteria. In a specific embodiment, a nucleic acid encoding an EphA2 antigenic peptide comprises a nucleotide sequence encoding a secretory signal, e.g., the SecA secretory signal or Tat signal, operatively linked to the nucleotide sequence

15   encoding the EphA2 antigenic peptide. A preferred strain of bacteria for use in the methods and compositions of the invention is *Pseudomonas aeruginosa*. In certain specific embodiments, the bacteria is not *Listeria*, and more preferably is not *Listeria monocytogenes*. In certain embodiments, the bacteria is not *Bacillus anthracis, Cholera, Bordetalla pertussis, Corynebacterium diphtheriae, E. coli, Borrelia burgdorfer* (Lyme),

20   *Streptococcus pneumoniae, Salmonella, Staphylococcus sp., Mycobacterium tuberculosis, Brucella abortus, Brucella melitensis, Haemophilus influenzae, Neisseria meningitides, Yersinia pestis, Shigella sp., Francisella tulraensis, or Streptococcus pyogenes.*

[0039]   Another preferred infectious agent for use as an EphA2 antigenic peptide expression vehicle in accordance with the methods and compositions of the invention is a

25   virus, for example a retrovirus, including but not limited to lentivirus, an adenovirus, an adeno-associated virus, or a herpes simplex virus. Preferred viruses for administration to human subjects are attenuated viruses.

[0040]   As an alternative to an infectious agent or nucleic acid, an EphA2 antigenic peptide expression vehicle can be a mammalian cell comprising a recombinant nucleic acid,

30   said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic peptide. Preferably, the mammalian cell is a human cell. Mammalian cells for use in the methods and compositions of the invention may be encapsulated within a membrane, for example a THERACYTE membrane, and/or administered by means of implantation.

[0041]   Compositions of the present invention useful as EphA2 vaccines also include

35   anti-idiotypes of anti-EphA2 antibodies. In certain specific embodiments, the EphA2

vaccines comprise anti-idiotypes of the anti-EphA2 monoclonal antibodies secreted by the

hybridoma clones deposited in the ATCC as PTA-4572, PTA-4573, and PTA-4574.

[0042]   With respect to EphA2 vaccines comprising sensitized antigen presenting

cells, such as macrophages and dendritic cells, in certain embodiments, the antigen

5   presenting cells are sensitized prior to their administration. For example, the antigen

presenting cells may be sensitized by a method comprising: contacting the cells with a

composition comprising one or more EphA2 antigenic peptides, and optionally comprising

one or more heat shock proteins such as hsp70, gp96, or hsp90, in an amount effective to

sensitize the cells. In a preferred embodiment, the antigen presenting cells are autologous

10   to the individual to whom they are administered; however, the cells need not be autologous.

[0043]   The compositions and methods of the present invention are useful in the

treatment, prevention and/or management of hyperproliferative diseases. In certain

embodiments, the hyperproliferative disease is cancer. In certain embodiments, the cancer

is of an epithelial cell origin and/or involves cells that overexpress EphA2 relative to non-

15   cancer cells having the tissue type of said cancer cells. In specific embodiments, the cancer

is a cancer of the skin, lung, colon, breast, ovarian, esophogeal, prostate, bladder or

pancreas or is a renal cell carcinoma or melanoma. In yet other embodiments, the cancer is

of a T cell origin. In specific embodiments, the cancer is a leukemia or a lymphoma. In yet

other embodiments, the hyperproliferative disorder is non-neoplastic. In specific

20   embodiments, the non-neoplastic hyperproliferative disorder is an epithelial cell disorder.

Exemplary non-neoplastic hyperproliferative disorders are asthma, chronic pulmonary

obstructive disease, fibrosis (e.g., lung, kidney, and heart fibrosis), bronchial hyper

responsiveness, psoriasis, and seborrheic dermatitis. In certain embodiments, the

hyperproliferative disease is an endothelial cell disorder.

25   [0044]   The EphA2 antigenic peptide for use in accordance with the methods and

compositions of the present invention may comprise full length EphA2 or an antigenic

fragment, analog, or derivative thereof. In certain embodiments, the EphA2 antigenic

peptide comprises the extracellular domain of EphA2 or the intracellular domain of EphA2.

In certain embodiments, the EphA2 antigenic peptide lacks the EphA2 transmembrane

30   domain. In certain embodiments, the EphA2 antigenic peptide comprises the EphA2

extracellular and intracellular domains and lacks the transmembrane domain of EphA2. In

certain embodiments, the EphA2 antigenic peptide comprises full length EphA2 or a

fragment thereof with a substitution of lysine to methionine at amino acid residue 646 of

EphA2. In certain embodiments, the EphA2 antigenic peptide comprises the extracellular

35   and intracellular domains of EphA2, lacks the transmembrane domain of EphA2 and has a

substitution of lysine to methionine at amino acid residue 646 of EphA2. In certain embodiments the EphA2 antigenic peptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

[0045]   A vaccine of the invention may have one or a plurality of EphA2 antigenic

5   peptides (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more EphA2 antigenic peptides, or 2-5, 2-10, 2-20, 10-20, or 15-25 EphA2 antigenic peptides), a plurality of EphA2 antigenic peptide expression vehicles (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more EphA2 antigenic peptide expression vehicles, or 2-5, 2-10, 2-20, 10-20, or 15-25 EphA2 antigenic peptide expression vehicles), or antigen presenting cells sensitized with a plurality of

10   EphA2 antigenic peptides. A vaccine of the invention may also have one or more classes of immune response-inducing or -mediating reagents, for example both an EphA2 antigenic peptide and an EphA2 antigenic peptide expression vehicle, both an EphA2 antigenic peptide and an antigen-presenting cell sensitized with an EphA2 antigenic peptide, or both an EphA2 antigenic peptide expression vehicle and an antigen-presenting cell sensitized

15   with an EphA2 antigenic peptide.

[0046]   The methods of the present invention encompass combination therapy with an EphA2 vaccine and one or more additional therapies, for example an additional anti-cancer therapy. In certain embodiments, the additional anti-cancer therapy is an agonistic EphA2 antibody, *i.e.*, antibody that binds to EphA2 and induces signaling and

20   phosphorylation of EphA2. In other embodiments, the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

[0047]   The vaccines of the invention can be administered by mucosal, intranasal, parenteral, intramuscular, or intraperitoneal routes. In a specific embodiment, the vaccines

25   of the invention are administered locally to the site of a disease, by, *e.g.*, implantation or intratumoral injection.

[0048]   In other embodiments, the EphA2 vaccines of the invention are used to treat, prevent and/or manage a non-cancer disease or disorder associated with cell hyperproliferation (*i.e.*, a non-neoplastic hyperproliferative disorder), such as but not

30   limited to asthma, chronic obstructive pulmonary disease, restenosis (smooth muscle and/or endothelial), psoriasis, etc. In preferred embodiments, the hyperproliferative cells are epithelial. In preferred embodiments, the hyperproliferative cells overexpress EphA2. In a preferred embodiment, some (*e.g.*, 5% or less, 10% or less, 15% or less, 20% or less, 25% or less, 30% or less, 35% or less, 40% or less, 45% or less, 50% or less, 55% or less, 60%

35   or less, 75% or less, or 85% or less) EphA2 is not bound to ligand as assessed by an assay

known in the art (*e.g.*, an immunoassay), either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in amount of EphA2 relative to EphA2-ligand.

[0049]   In yet other aspects of the invention, the EphA2 vaccines are used to treat, prevent and/or manage a disorder associated with or involving aberrant angiogenesis. The

5   EphA2 vaccines are used to elicit an immune response against EphA2 expressed on neovasculature. Thus, the present invention provides methods of treating, preventing and/or managing a disorder associated with or involving aberrant angiogenesis comprising administering to a subject in need thereof a composition comprising an EphA2 vaccine in an amount effective to treat, prevent and/or manage the disorder associated with or

10   involving aberrant angiogenesis. Examples of such disorders include but are not limited to macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome,

15   endometriosis, preeclampsia, atherosclerosis and coronary artery disease.

[0050]   The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental cancer therapies, including but not limited to chemotherapies, hormonal therapies, biological therapies, radiation therapies, and/or

20   surgery as well as to improve the efficacy of such treatments. In particular, EphA2 expression has been implicated in increasing levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In addition, EphA2 overexpression can override the need for estrogen receptor activity thus contributing to tamoxifen resistance in

25   breast cancer cells. Accordingly, in a preferred embodiment, the invention provides therapeutic and prophylactic methods for the treatment, prevention or management of cancer that has been shown to be or may be refractory or non-responsive to therapies other than those comprising administration of EphA2 antibodies of the invention. In a specific embodiment, one or more EphA2 vaccines of the invention are administered to a patient

30   refractory or non-responsive to a non-EphA2-based treatment, particularly tamoxifen treatment or a treatment in which resistance is associated with increased IL-6 levels, to render the patient non-refractory or responsive. The treatment to which the patient had previously been refractory or non-responsive can then be administered with therapeutic effect.

[0051]   The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental therapies for non-neoplastic hyperproliferative disorders and/or disorders associated with or involving aberrant angiogenesis. The methods and compositions of the invention are useful for the treatment of patients partially or completely refractory to current standard and experimental therapies for neoplastic hyperproliferative disorders and/or disorders associated with or involving aberrant angiogenesis (e.g., macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis and coronary artery disease), asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis).

[0052]   In certain embodiments of the invention, antibodies are generated by administering an EphA2 vaccine to a subject (e.g., a mouse) and harvesting the antibodies. The antibodies generated may be modified, e.g., to increase affinity for an antigen, increase the half-life, or to humanize. The antibodies generated can be used for passive immunotherapy, for diagnostic immunoassays and/or the generation of anti-idiotypic antibodies.

[0053]   The present invention also provides kits comprising the vaccines or vaccine components of the invention.

3.1 DEFINITIONS

[0054]   As used herein, the term "EphA2 vaccine" can be any reagent that elicits or mediates an immune response against EphA2 on hyperproliferative cells. In certain embodiments, an EphA2 vaccine is an EphA2 antigenic peptide of the invention, an expression vehicle (e.g., a naked nucleic acid or a viral or bacterial vector or a cell) for an EphA2 antigenic peptide (e.g., which delivers the EphA2 antigenic peptide), or T cells or antigen presenting cells (e.g., dendritic cells or macrophages) that have been primed with the EphA2 antigenic peptide of the invention.

[0055]   As used herein, the terms "EphA2 antigenic peptide" and "EphA2 antigenic polypeptide" refer to an EphA2 polypeptide, preferably of SEQ ID NO:2, or a fragment, analog, or derivative thereof comprising one or more B cell epitopes or T cell epitopes of EphA2. The EphA2 polypeptide may be from any species. In certain embodiments, an

EphA2 polypeptide refers to the mature, processed form of EphA2. In other embodiments, an EphA2 polypeptide refers to an immature form of EphA2.

[0056]   The nucleotide and/or amino acid sequences of EphA2 polypeptides can be found in the literature or public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing techniques known to one of skill in the art. For example, the nucleotide sequence of human EphA2 can be found in the GenBank database (see, *e.g.*, Accession Nos. BC037166, M59371 and M36395). The amino acid sequence of human EphA2 can be found in the GenBank database (see, *e.g.*, Accession Nos. NP_004422, AAH37166 and AAA53375). Additional non-limiting examples of amino acid sequences of EphA2 are listed in Table 1, *infra*.

[0057]   Table 1

Species	GenBank Accession No.
Mouse	NP_034269, AAH06954
Rat	XP_345597
Chicken	BAB63910

[0058]   In certain embodiments, the EphA2 antigenic peptides are not one or more of the following peptides: TLADFDPRV (SEQ ID NO:3); VLLLVLAGV (SEQ ID NO:4); VLAGVGFFI (SEQ ID NO:5); IMNDMPIYM (SEQ ID NO:6); SLLGLKDQV (SEQ ID NO:7); WLVPIGQCL (SEQ ID NO:8); LLWGCALAA (SEQ ID NO:9); GLTRTSVTV (SEQ ID NO:10); NLYYAESDL (SEQ ID NO:11); KLNVEERSV (SEQ ID NO:12); IMGQFSHHN (SEQ ID NO:13); YSVCNVMSG (SEQ ID NO:14); MQNIMNDMP (SEQ ID NO:15); EAGIMGQFSHHNIIR (SEQ ID NO:16); PIYMYSVCNVMSG (SEQ ID NO:17); DLMQNIMNDMPIYMYS (SEQ ID NO:18). In certain specific embodiments, the EphA2 antigenic peptide is not any of SEQ ID NO:3-12, is not SEQ ID NO:13-15, and/or is not SEQ ID NO:16-18. In yet another specific embodiment, the EphA2 antigenic peptide is not SEQ ID NO:3-18.

[0059]   As used herein, the term "analog" in the context of a proteinaceous agent (*e.g.*, a peptide, polypeptide, protein or antibody) refers to a proteinaceous agent that possesses a similar or identical function as a second proteinaceous agent (*e.g.*, an EphA2 polypeptide) but does not necessarily comprise a similar or identical amino acid sequence or structure of the second proteinaceous agent. A proteinaceous agent that has a similar amino acid sequence refers to a proteinaceous agent that satisfies at least one of the following: (a) a proteinaceous agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical

to the amino acid sequence of a second proteinaceous agent; (b) a proteinaceous agent

encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide

sequence encoding a second proteinaceous agent of at least 20 amino acid residues, at least

30 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at

5   least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at

least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid

residues, or at least 150 amino acid residues; and (c) a proteinaceous agent encoded by a

nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least

50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at

10   least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence

encoding a second proteinaceous agent. A proteinaceous agent with similar structure to a

second proteinaceous agent refers to a proteinaceous agent that has a similar secondary,

tertiary or quaternary structure of the second proteinaceous agent. The structure of a

proteinaceous agent can be determined by methods known to those skilled in the art,

15   including but not limited to, X-ray crystallography, nuclear magnetic resonance, and

crystallographic electron microscopy. Preferably, the proteinaceous agent has EphA2

activity.

[0060]   To determine the percent identity of two amino acid sequences or of two

nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*,

20   gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for

optimal alignment with a second amino acid or nucleic acid sequence). The amino acid

residues or nucleotides at corresponding amino acid positions or nucleotide positions are

then compared. When a position in the first sequence is occupied by the same amino acid

residue or nucleotide as the corresponding position in the second sequence, then the

25   molecules are identical at that position. The percent identity between the two sequences is a

function of the number of identical positions shared by the sequences (*i.e.*, % identity =

number of identical overlapping positions/total number of positions x 100%). In one

embodiment, the two sequences are the same length.

[0061]   The determination of percent identity between two sequences can also be

30   accomplished using a mathematical algorithm. A preferred, non-limiting example of a

mathematical algorithm utilized for the comparison of two sequences is the algorithm of

Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87: 2264-2268, modified as in

Karlin and Altschul, 1993 , *Proc. Natl. Acad. Sci. U.S.A.* 90: 5873-5877. Such an algorithm

is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, *J. Mol.*

35   *Biol.* 215: 403. BLAST nucleotide searches can be performed with the NBLAST

nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide

sequences homologous to a nucleic acid molecules of the present invention. BLAST

protein searches can be performed with the XBLAST program parameters set, *e.g.*, to

score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule

5   of the present invention. To obtain gapped alignments for comparison purposes, Gapped

BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-

3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects

distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST,

and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of

10   XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred,

non-limiting example of a mathematical algorithm utilized for the comparison of sequences

is the algorithm of Myers and Miller, 1988, *CABIOS* 4: 11-17. Such an algorithm is

incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence

alignment software package. When utilizing the ALIGN program for comparing amino

15   acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap

penalty of 4 can be used.

[0062]   The percent identity between two sequences can be determined using

techniques similar to those described above, with or without allowing gaps. In calculating

percent identity, typically only exact matches are counted.

20   [0063]   As used herein, the term "analog" in the context of a non-proteinaceous

analog refers to a second organic or inorganic molecule which possesses a similar or

identical function as a first organic or inorganic molecule and is structurally similar to the

first organic or inorganic molecule.

[0064]   As used herein, the terms "attenuated" and "attenuation" refer to a

25   modification(s) so that the bacteria are less pathogenic. The end result of attenuation is that

the risk of toxicity as well as other side effects is decreased when the bacteria are

administered to a subject.

[0065]   As used herein, the term "derivative" in the context of a proteinaceous agent

(*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that

30   comprises the amino acid sequence which has been altered by the introduction of amino

acid residue substitutions, deletions, and/or additions. The term "derivative" as used herein

also refers to a proteinaceous agent which has been modified, *i.e.*, by the covalent

attachment of a type of molecule to the proteinaceous agent. For example, but not by way

of limitation, a derivative of a proteinaceous agent may be produced, *e.g.*, by glycosylation,

35   acetylation, pegylation, phosphorylation, amidation, derivatization by known

protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other

protein, etc. A derivative of a proteinaceous agent may also be produced by chemical

modifications using techniques known to those of skill in the art, including, but not limited

to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin,

5   etc. Further, a derivative of a proteinaceous agent may contain one or more non-classical

amino acids. A derivative of a proteinaceous agent possesses an identical function(s) as the

proteinaceous agent from which it was derived.

[0066]   As used herein, the term "derivative" in the context of EphA2 proteinaceous

agents refers to a proteinaceous agent that comprises an amino acid sequence of an EphA2

10   polypeptide or a fragment of an EphA2 polypeptide that has been altered by the

introduction of amino acid residue substitutions, deletions or additions (i.e., mutations).

The term "derivative" as used herein in the context of EphA2 proteinaceous agents also

refers to an EphA2 polypeptide or a fragment of an EphA2 polypeptide which has been

modified, i.e, by the covalent attachment of any type of molecule to the polypeptide. For

15   example, but not by way of limitation, an EphA2 polypeptide or a fragment of an EphA2

polypeptide may be modified, e.g., by glycosylation, acetylation, pegylation,

phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of an

EphA2 polypeptide or a fragment of an EphA2 polypeptide may be modified by chemical

20   modifications using techniques known to those of skill in the art, including, but not limited

to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of

tunicamycin, etc. Further, a derivative of an EphA2 polypeptide or a fragment of an EphA2

polypeptide may contain one or more non-classical amino acids. In one embodiment, a

polypeptide derivative possesses a similar or identical function as an EphA2 polypeptide or

25   a fragment of an EphA2 polypeptide described herein. In another embodiment, a derivative

of EphA2 polypeptide or a fragment of an EphA2 polypeptide has an altered activity when

compared to an unaltered polypeptide. For example, a derivative of an EphA2 polypeptide

or fragment thereof can differ in phosphorylation relative to an EphA2 polypeptide or

fragment thereof.

30   [0067]   As used herein, the term "derivative" in the context of a non-proteinaceous

agent refers to a second organic or inorganic molecule that is formed based upon the

structure of a first organic or inorganic molecule. A derivative of an organic molecule

includes, but is not limited to, a molecule modified, e.g., by the addition or deletion of a

hydroxyl, methyl, ethyl, carboxyl, nitryl, or amine group. An organic molecule may also,

35   for example, be esterified, alkylated and/or phosphorylated.

[0068]   As used herein, the term "EphrinA1 polypeptide" refers to EphrinA1, an

analog, derivative or a fragment thereof, or a fusion protein comprising EphrinA1, an

analog, derivative or a fragment thereof. The EphrinA1 polypeptide may be from any

species. In certain embodiments, the term "EphrinA1 polypeptide" refers to the mature,

5   processed form of EphrinA1. In other embodiments, the term "EphrinA1 polypeptide"

refers to an immature form of EphrinA1. In accordance with this embodiment, the

antibodies of the invention immunospecifically bind to the portion of the immature form of

EphrinA1 that corresponds to the mature, processed form of EphrinA1.

[0069]   The nucleotide and/or amino acid sequences of EphrinA1 polypeptides can

10   be found in the literature or public databases, or the nucleotide and/or amino acid sequences

can be determined using cloning and sequencing techniques known to one of skill in the art.

For example, the nucleotide sequence of human EphrinA1 can be found in the GenBank

database (see, *e.g.*, Accession No. BC032698). The amino acid sequence of human

EphrinA1 can be found in the GenBank database (see, *e.g.*, Accession No. AAH32698).

15   Additional non-limiting examples of amino acid sequences of EphrinA1 are listed in Table

2, *infra*.

Table 2

Species	GenBank Accession No.
Mouse	NP_034237
Rat	NP_446051

20   [0070]   In a specific embodiment, a EphrinA1 polypeptide is EphrinA1 from any

species. In a preferred embodiment, an EphrinA1 polypeptide is human EphrinA1.

[0071]   As used herein, the term "effective amount" refers to the amount of a therapy

(*e.g.*, a prophylactic or therapeutic agent) which is sufficient to reduce and/or ameliorate the

severity and/or duration of a disorder (*e.g.*, cancer, a non-neoplastic hyperproliferative cell

25   disorder or a disorder associated with aberrant angiogenesis) or a symptom thereof, prevent

the advancement of said disorder, cause regression of said disorder, prevent the recurrence,

development, or onset of one or more symptoms associated with said disorder, or enhance

or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or

therapeutic agent).

30   [0072]   As used herein, the term "B cell epitope" refers to a portion of an EphA2

polypeptide having antigenic or immunogenic activity in an animal, preferably in a

mammal, and most preferably in a mouse or a human. An epitope having immunogenic

activity is a portion of an EphA2 polypeptide that elicits an antibody response in an animal.

An epitope having antigenic activity is a portion of an EphA2 polypeptide to which an

antibody immunospecifically binds as determined by any method well known in the art, for

example, by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

[0073]   As used herein, the term "T cell epitope" refers to at least a portion of an

EphA2 polypeptide, preferably an EphA2 polypeptide of SEQ ID NO:2, that is recognized

by a T cell receptor. The term "T cell epitope" encompasses helper T cell (Th) epitopes and

cytotoxic T cell (Tc) epitopes. The term "helper T cell epitopes" encompasses Th1 and Th2

epitopes.

[0074]   As used herein, the term "fragments" in the context of EphA2 polypeptides

include an EphA2 antigenic peptide or polypeptide comprising an amino acid sequence of at

least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least

15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25

contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50

contiguous amino acid residues, at least 60 contiguous amino residues, at least 70

contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90

contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125

contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175

contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250

contiguous amino acid residues of the amino acid sequence of an EphA2 polypeptide.

[0075]   As used herein, the term "fusion protein" refers to a polypeptide or protein

that comprises the amino acid sequence of a first polypeptide or protein or fragment, analog

or derivative thereof, and the amino acid sequence of a heterologous polypeptide or protein.

In one embodiment, a fusion protein comprises a prophylactic or therapeutic agent fused to

a heterologous protein, polypeptide or peptide. In accordance with this embodiment, the

heterologous protein, polypeptide or peptide may or may not be a different type of

prophylactic or therapeutic agent. For example, two different proteins, polypeptides, or

peptides with immunomodulatory activity may be fused together to form a fusion protein.

In a preferred embodiment, fusion proteins retain or have improved activity relative to the

activity of the original polypeptide or protein prior to being fused to a heterologous protein,

polypeptide, or peptide.

[0076]   As used herein, the term "heterologous," in the context of a nucleic acid

sequence (e.g., a gene) or an amino acid sequence (e.g., a peptide, polypeptide or protein)

refers a nucleic acid sequence or an amino acid sequence that is not found in nature to be

associated with a second nucleic acid sequence or a second amino acid sequence (e.g., a

nucleic acid sequence or an amino acid sequence derived from a different species).

[0077]   As used herein, the term "host cell" includes a particular subject cell transfected or transformed with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0078]   As used herein, the terms "hyperproliferative cell disorder," "hyperproliferative cell disease," "hyperproliferative disorder," and "hyperproliferative disease" and analogous terms refer to a disorder in which cellular hyperproliferation or any form of excessive cell accumulation causes or contributes to the pathological state or symptoms of the disorder. In some embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating epithelial cells. In other embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating endothelial cells. In other embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating fibroblasts. In certain embodiments, the hyperproliferative cell disorder is not neoplastic. Exemplary non-neoplastic hyperproliferative cell disorders are asthma, chronic pulmonary obstructive disease, fibrosis (e.g., lung, liver, and kidney fibrosis), bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis. In a preferred embodiment, the hyperproliferative cell disorder is characterized by hyperproliferating cells that express (preferably, overexpress) EphA2.

[0079]   As used herein, the term "immunospecifically binds to an antigen" and analogous terms refer to peptides, polypeptides, proteins, fusion proteins and antibodies or fragments thereof that specifically bind to an antigen or a fragment and do not specifically bind to other antigens. A peptide, polypeptide, protein, or antibody that immunospecifically binds to an antigen may bind to other peptides, polypeptides, or proteins with lower affinity as determined by, e.g., immunoassays, BIAcore, or other assays known in the art. Antibodies or fragments that immunospecifically bind to an antigen may be cross-reactive with related antigens. Preferably, antibodies or fragments that immunospecifically bind to an antigen do not cross-react with other antigens. An antibody binds specifically to an antigen when it binds to the antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). See, e.g., Paul, ed., 1989, Fundamental Immunology, 2nd ed., Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity.

[0080]   As used herein, the term "immunospecifically binds to EphA2" and
analogous terms refers to peptides, polypeptides, proteins, fusion proteins, and antibodies or
fragments thereof that specifically bind to an EphA2 receptor or one or more fragments
thereof and do not specifically bind to other receptors or fragments thereof. The terms
"immunospecifically binds to EphrinA1" and analogous terms refer to peptides,
polypeptides, proteins, fusion proteins, and antibodies or fragments thereof that specifically
bind to EphrinA1 or one or more fragments thereof and do not specifically bind to other
ligands or fragments thereof. A peptide, polypeptide, protein, or antibody that
immunospecifically binds to EphA2 or EphrinA1, or fragments thereof, may bind to other
peptides, polypeptides, or proteins with lower affinity as determined by, e.g., immunoassays
or other assays known in the art to detect binding affinity. Antibodies or fragments that
immunospecifically bind to EphA2 or EphrinA1 may be cross-reactive with related
antigens. Preferably, antibodies or fragments thereof that immunospecifically bind to
EphA2 or EphrinA1 can be identified, for example, by immunoassays or other techniques
known to those of skill in the art. An antibody or fragment thereof binds specifically to
EphA2 or EphrinA1 when it binds to EphA2 or EphrinA1 with higher affinity than to any
cross-reactive antigen as determined using experimental techniques, such as
radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). See, e.g.,
Paul, ed., 1989, Fundamental Immunology, 2nd ed., Raven Press, New York at pages 332-
336 for a discussion regarding antibody specificity. In a preferred embodiment, an antibody
that immunospecifically binds to EphA2 or EphrinA1 does not bind or cross-react with
other antigens. In another embodiment, an antibody that binds to EphA2 or EphrinA1 that
is a fusion protein specifically binds to the portion of the fusion protein that is EphA2 or
EphrinA1.

[0081]   Antibodies of the invention include, but are not limited to, synthetic
antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific
antibodies (including bi-specific antibodies), human antibodies, humanized antibodies,
chimeric antibodies, intrabodies, single-chain Fvs (scFv) (e.g., including monospecific and
bi-specific, etc.), Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-
idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. In
particular, antibodies of the present invention include immunoglobulin molecules and
immunologically active portions of immunoglobulin molecules, i.e., molecules that contain
an antigen-binding site that immunospecifically binds to an EphA2 antigen or an EphrinA1
antigen (e.g., one or more complementarity determining regions (CDRs) of an anti-EphA2
antibody or of an anti-EphrinA1 antibody). The antibodies of the invention can be of any

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type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG$_1$, IgG$_2$, IgG$_3$, IgG$_4$, IgA$_1$ and IgA$_2$) or subclass of immunoglobulin molecule.

[0082]   As used herein, the term "isolated" in the context of an organic or inorganic molecule (whether it be a small or large molecule), other than a proteinaceous agent or a nucleic acid, refers to an organic or inorganic molecule substantially free of a different organic or inorganic molecule. Preferably, an organic or inorganic molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% free of a second, different organic or inorganic molecule. In a preferred embodiment, an organic and/or inorganic molecule is isolated.

[0083]   As used herein, the term "isolated" in the context of a proteinaceous agent (*e.g.*, a peptide, polypeptide, fusion protein, or antibody) refers to a proteinaceous agent which is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a proteinaceous agent in which the proteinaceous agent is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a proteinaceous agent that is substantially free of cellular material includes preparations of a proteinaceous agent having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein, polypeptide, peptide, or antibody (also referred to as a "contaminating protein"). When the proteinaceous agent is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the proteinaceous agent preparation. When the proteinaceous agent is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the proteinaceous agent. Accordingly, such preparations of a proteinaceous agent have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the proteinaceous agent of interest. In a specific embodiment, proteinaceous agents disclosed herein are isolated.

[0084]   As used herein, the term "isolated" in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, is preferably substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, nucleic acid molecules are isolated.

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[0085]   As used herein, the term "disease" and "disorder" are used interchangeably to refer to a condition.

[0086]   As used herein, the term "in combination" refers to the use of more than one therapies (*e.g.*, prophylactic and/or therapeutic agents). The use of the term "in

5   combination" does not restrict the order in which therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject with a hyperproliferative cell disorder, especially cancer. A first therapy (*e.g.*, prophylactic and/or therapeutic agent) can be administered prior to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2

10   weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (*e.g.*, prophylactic and/or therapeutic agent) to a subject which had, has,

15   or is susceptible to a hyperproliferative cell disorder, especially cancer. The therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject in a sequence and within a time interval such that the agent of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. Any additional therapy (*e.g.*, prophylactic and/or therapeutic agent) can be administered in any

20   order with the other additional therapy (*e.g.*, prophylactic and/or therapeutic agent).

[0087]   As used herein, the phrase "low tolerance" refers to a state in which the patient suffers from side effects from treatment so that the patient does not benefit from and/or will not continue therapy because of the adverse effects and/or the harm from the side effects outweighs the benefit of the treatment.

25   [0088]   As used herein, the terms "manage," "managing" and "management" refer to the beneficial effects that a subject derives from administration of a therapy (*e.g.*, prophylactic and/or therapeutic agent), which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more therapies (*e.g.*, prophylactic and/or therapeutic agents) to "manage" a disease so as to prevent the progression or

30   worsening of the disease.

[0089]   As used herein, the term "neoplastic" refers to a disease involving cells that have the potential to metastasize to distal sites and exhibit phenotypic traits that differ from those of non-neoplastic cells, for example, formation of colonies in a three-dimensional substrate such as soft agar or the formation of tubular networks or weblike matrices in a

35   three-dimensional basement membrane or extracellular matrix preparation, such as

MATRIGEL™. Non-neoplastic cells do not form colonies in soft agar and form distinct sphere-like structures in three-dimensional basement membrane or extracellular matrix preparations. Neoplastic cells acquire a characteristic set of functional capabilities during their development, albeit through various mechanisms. Such capabilities include evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion/metastasis, limitless replicative potential, and sustained angiogenesis. Thus, "non-neoplastic" means that the condition, disease, or disorder does not involve cancer cells.

[0090]   As used herein, the phrase "non-responsive/refractory" is used to describe patients treated with one or more currently available therapies (*e.g.*, cancer therapies) such as chemotherapy, radiation therapy, surgery, hormonal therapy and/or biological therapy/immunotherapy, particularly a standard therapeutic regimen for the particular cancer, wherein the therapy is not clinically adequate to treat the patients such that these patients need additional effective therapy, *e.g.*, remain unsusceptible to therapy. The phrase can also describe patients who respond to therapy yet suffer from side effects, relapse, develop resistance, *etc.* In various embodiments, "non-responsive/refractory" means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are "non-responsive/refractory" can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is "non-responsive/refractory" where the number of cancer cells has not been significantly reduced, or has increased during the treatment.

[0091]   As used herein, the term "overexpress" in the context of EphA2 overexpression means that the gene encoding EphA2 is expressed at a level above that which is expressed by a normal human cell as assessed by an assay described herein or known to one of skill in the art (*e.g.*, an immunoassay such as an ELISA or Western blot, a Northern blot, or RT-PCR).

[0092]   As used herein, the term "potentiate" refers to an improvement in the efficacy of a therapy at its common or approved dose.

[0093]   As used herein, the terms "prevent," " preventing" and "prevention" refer to the prevention of the onset, recurrence, or spread of a disease in a subject resulting from the administration of a therapy (*e.g.*, prophylactic or therapeutic agent), or a combination of therapies.

[0094]   As used herein, the term "prophylactic agent" refers to any agent that can be used in the prevention of the onset, recurrence or spread of a disease associated with EphA2 overexpression, a disorder associated with aberrant angiogenesis and/or a hyperproliferative

cell disease, particularly cancer. In certain embodiments, the term "prophylactic agent" refers to an EphA2 vaccine of the invention, such as a composition comprising an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, or an antigen presenting cell sensitized with an EphA2 antigenic peptide. In certain other embodiments, the term

5   "prophylactic agent" refers to a therapy other than an EphA2 vaccine, *e.g.*, a cancer chemotherapeutic, radiation therapy, hormonal therapy, biological therapy (*e.g.*, immunotherapy). In other embodiments, more than one prophylactic agent may be administered in combination.

[0095]   As used herein, a "prophylactically effective amount" refers to that amount

10   of a therapy (*e.g.*, a prophylactic agent) sufficient to result in the prevention of the onset, recurrence or spread of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease, preferably, cancer). A prophylactically effective amount may refer to the amount of therapy (*e.g.*, a prophylactic agent) sufficient to prevent the onset, recurrence or spread of a disorder (*e.g.*, a disorder associated with aberrant

15   angiogenesis and a hyperproliferative cell disease, particularly cancer) in a subject including, but not limited to, subjects predisposed to a hyperproliferative cell disease, for example, those genetically predisposed to cancer or previously exposed to carcinogens. A prophylactically effective amount may also refer to the amount of a therapy (*e.g.*, prophylactic agent) that provides a prophylactic benefit in the prevention of a disorder (*e.g.*,

20   a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease). Further, a prophylactically effective amount with respect to a therapy (*e.g.*, prophylactic agent) means that amount of a therapy (*e.g.*, prophylactic agent) alone, or in combination with other therapies (*e.g.*, agents), that provides a prophylactic benefit in the prevention of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell

25   disease). Used in connection with an amount of an EphA2 vaccine of the invention, the term can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of or synergies with another therapy (*e.g.*, a prophylactic agent).

[0096]   As used herein, a "protocol" includes dosing schedules and dosing regimens.

[0097]   As used herein, the phrase "side effects" encompasses unwanted and adverse

30   effects of a prophylactic or therapeutic agent. Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a therapy (e.g., a prophylactic or therapeutic agent) might be harmful or uncomfortable or risky. Side effects from chemotherapy include, but are not limited to, gastrointestinal toxicity such as, but not limited to, early and late-forming diarrhea and flatulence, nausea, vomiting, anorexia,

35   leukopenia, anemia, neutropenia, asthenia, abdominal cramping, fever, pain, loss of body

weight, dehydration, alopecia, dyspnea, insomnia, dizziness, mucositis, xerostomia, and kidney failure, as well as constipation, nerve and muscle effects, temporary or permanent damage to kidneys and bladder, flu-like symptoms, fluid retention, and temporary or permanent infertility. Side effects from radiation therapy include but are not limited to

5   fatigue, dry mouth, and loss of appetite. Side effects from biological therapies/immunotherapies include but are not limited to rashes or swellings at the site of administration, flu-like symptoms such as fever, chills and fatigue, digestive tract problems and allergic reactions. Side effects from hormonal therapies include but are not limited to nausea, fertility problems, depression, loss of appetite, eye problems, headache, and weight

10   fluctuation. Additional undesired effects typically experienced by patients are numerous and known in the art. Many are described in the *Physicians' Desk Reference* (56th ed., 2002, 57th ed., 2003 and 58th ed., 2004).

[0098]   As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, a subject is preferably a mammal such as a non-primate (*e.g.*, cows, pigs,

15   horses, cats, dogs, rats *etc.*) and a primate (*e.g.*, monkey and human), most preferably a human. In a specific embodiment, the subject is a non-human animal. In another embodiment, the subject is a farm animal (*e.g.*, a horse, a pig, a lamb or a cow) or a pet (*e.g.*, a dog, a cat, a rabbit or a bird). In another embodiment, the subject is an animal other than a laboratory animal or animal model (*e.g.*, a mouse, a rat, a guinea pig or a monkey).

20   In a preferred embodiment, the subject is a human. In another preferred embodiment, the subject is a human that is not immunocompromised or immunosuppressed. In another preferred embodiment, the subject is a human with a mean absolute lymphocyte count of approximately 500 cells/mm^3, approximately 600 cells/mm^3, approximately 650 cells/mm^3, approximately 700 cells/mm^3, approximately 750 cells/mm^3, approximately 800 cells/mm^3,

25   approximately 850 cells/mm^3, approximately 900 cells/mm^3, approximately 950 cells/mm^3, approximately 1000 cells/mm^3, approximately 1050 cells/mm^3, approximately 1100 cells/mm^3, or approximately 1150 cells/mm^3 or approximately 1200 cells/mm^3.

[0099]   As used herein, the terms "treat," "treating" and "treatment" refer to the eradication, reduction or amelioration of a disorder or a symptom thereof, particularly, the

30   eradication, removal, modification, or control of primary, regional, or metastatic cancer tissue that results from the administration of one or more therapies (*e.g.*, therapeutic agents). In certain embodiments, such terms refer to the minimizing or delaying the spread of cancer resulting from the administration of one or more therapies (*e.g.*, therapeutic agents) to a subject with such a disease.

[00100]   As used herein, the term "therapeutic agent" refers to any agent that can be used in the prevention, treatment, or management of a disease (*e.g.*, a disorder associated with overexpression of EphA2 and/or hyperproliferative cell disorder, particularly, cancer). In certain embodiments, the term "therapeutic agent" refers to an EphA2 vaccine of the invention, such as a composition comprising an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, or an antigen presenting cell sensitized with an EphA2 antigenic peptide. In certain other embodiments, the term "therapeutic agent" refers to a therapy other than a EphA2 vaccine such as, e.g., a cancer chemotherapeutic, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy. In other embodiments, more than one therapy (e.g., a therapeutic agent) may be administered in combination.

[00101]   As used herein, a "therapeutically effective amount" refers to that amount of a therapy (*e.g.*, a therapeutic agent) sufficient to treat or manage a disorder (*e.g.*, a disorder associated with EphA2 overexpression, a disorder associated with aberrant angiogenesis and/or hyperproliferative cell disease) and, preferably, the amount sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue. A therapeutically effective amount may refer to the amount of a therapy (*e.g.*, a therapeutic agent) sufficient to delay or minimize the onset of a disorder (*e.g.*, hyperproliferative cell disease), *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of a therapy (*e.g.*, a therapeutic agent) that provides a therapeutic benefit in the treatment or management of a disorder (*e.g.*, cancer). Further, a therapeutically effective amount with respect to a therapy (*e.g.*, a therapeutic agent) means that amount of a therapy (*e.g.*, therapeutic agent) alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of a disorder (*e.g.*, a hyperproliferative cell disease such as cancer). Used in connection with an amount of an EphA2 vaccine of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy (*e.g.*, a therapeutic agent).

[00102]   As used herein, the term "therapy" refers to any protocol, method and/or agent that can be used in the prevention, treatment or management of a disorder (*e.g.*, a hyperproliferative cell disorder, a disorder associated with aberrant angiogenesis and/or a non-neoplastic hyperproliferative cell disorder) or a symptom thereof. In certain embodiments, the terms "therapies" and "therapy" refer to a biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration of a disorder (*e.g.*, a hyperproliferative cell disorder and/or a non-neoplastic

hyperproliferative cell disorder) or one or more symptoms thereof known to one of skill in the art such as medical personnel.

[00103]   As used herein, the term "synergistic" refers to a combination of therapies (*e.g.*, prophylactic or therapeutic agents) which is more effective than the additive effects of

5   any two or more single therapies (*e.g.*, one or more prophylactic or therapeutic agents). A synergistic effect of a combination of therapies (*e.g.*, a combination of prophylactic or therapeutic agents) permits the use of lower dosages of one or more of therapies (*e.g.*, one or more prophylactic or therapeutic agents) and/or less frequent administration of said therapies to a subject with a non-neoplastic hyperproliferative epithelial and/or endothelial

10   cell disorder. The ability to utilize lower dosages of therapies (*e.g.*, prophylactic or therapeutic agents) and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention or treatment of a disorder (*e.g.*, a hyperproliferative cell disorder). In addition, a synergistic effect can result in improved

15   efficacy of therapies (*e.g.*, prophylactic or therapeutic agents) in the prevention or treatment of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disorder). Finally, synergistic effect of a combination of therapies (*e.g.*, prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of any single therapy. In a preferred embodiment, the combination of an EphA2

20   vaccine and a therapy other than an EphA2 vaccine results in synergistic effect.

[00104]   As used herein, the terms "T cell malignancies" and "T cell malignancy" refer to any T cell lymphoproliferative disorder, including thymic and post-thymic malignancies. T cell malignancies include tumors of T cell origin. T cell malignancies refer to tumors of lymphoid progenitor cell, thymocyte, T cell, NK-cell, or antigen

25   presenting cell origin. T cell malignancies include, but are not limited to, leukemias, including acute lymphoblastic leukemias, thymomas, acute lymphoblastic leukemias, and lymphomas, including Hodgkin's and non-Hodgkin's disease, with the proviso that T cell malignancies are not cutaneous T cell malignancies, in particular cutaneous-cell lymphomas. In a preferred embodiment, T cell malignancies are systemic, non-cutaneous T

30   cell malignancies.

3.2 SEQUENCES

[00105]   Below is a brief summary of the sequences presented in the accompanying sequence listing, which is incorporated by reference herein in its entirety:

[00106]   **SEQ ID NO:1**

35   Human EphA2 cDNA (full length)

[00107]   **SEQ ID NO:2**

Human EphA2 polypeptide (full length)

[00108]   **SEQ ID NOs:3-18**

Human EphA2 peptides

5   [00109]   **SEQ ID NO:19**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

10   Fusion protein coding sequence shown

[00110]   **SEQ ID NO:20**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

15   No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00111]   **SEQ ID NO:21**

EphA2 EX2 domain

Native nucleotide sequence

20   [00112]   **SEQ ID NO:22**

EphA2 EX2 domain

Nucleotide sequence for optimal codon usage in Listeria

[00113]   **SEQ ID NO:23**

EphA2 EX2 domain

25   Primary Amino Acid Sequence

[00114]   **SEQ ID NO:24**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human
        EphA2

30   Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

[00115]   **SEQ ID NO:25**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human

35   EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00116]   **SEQ ID NO:26**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -

     FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00117]   **SEQ ID NO:27**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -

     FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[00118]   **SEQ ID NO:28**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon

     optimized -FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00119]   **SEQ ID NO:29**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon

     optimized -FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[00120]   **SEQ ID NO:30**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-

     Myc)

Nucleotide Sequence (including *hly* promoter)

[00121]   **SEQ ID NO:31**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-

     Myc)

Amino acid sequence

[00122]   **SEQ ID NO:32**

EphA2 CO domain

Native nucleotide sequence

[00123] **SEQ ID NO:33**

EphA2 CO domain

Nucleotide sequence for optimal codon usage in Listeria

5 [00124] **SEQ ID NO:34**

EphA2 CO domain

Primary Amino Acid Sequence

[00125] **SEQ ID NO:35**

Construct: LLOss-PEST-CO-huEphA2

10 Native LLO signal peptide + PEST fused to cytoplasmic domain of human

EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Fusion protein coding sequence shown

15 [00126] **SEQ ID NO:36**

Construct: LLOss-PEST-CO-huEphA2

Native LLO signal peptide + PEST fused to cytoplasmic domain of human

EphA2

Not Codon optimized

20 No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00127] **SEQ ID NO:37**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -

25 FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00128] **SEQ ID NO:38**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -

30 FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[00129] **SEQ ID NO:39**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon

35 optimized -FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00130]   **SEQ ID NO:40**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon

optimized -FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[00131]   **SEQ ID NO:41**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-

Myc)

Nucleotide Sequence (including *hly* promoter)

[00132]   **SEQ ID NO:42**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-

Myc)

Amino acid sequence

[00133]   **SEQ ID NO:43**

Construct: pAM401-MCS

Plasmid pAM401 containing multiple cloning site (MCS) from pPL2 vector

Insertion of small *Aat II* MCS fragment from pPL2 inserted into pAM401

plasmid between blunted *Xba I* and *Nru I* sites.

Complete pAM401-MCS plasmid sequence shown

4.   BRIEF DESCRIPTION OF THE FIGURES

[00134]   **Figure 1.** *Listeria* intracellular life cycle, antigen presenting cell activation,
and antigen presentation.

[00135]   **Figure 2.** Western blot analysis of secreted protein from recombinant
Listeria encoding native EphA2 CO domain sequence.

[00136]   **Figure 3.** Western blot analysis of secreted protein from recombinant
Listeria encoding native or codon-optimized LLO secA1 signal peptide fused with codon-
optimized EphA2 EX2 domain sequence signal peptide.

[00137]   **Figure 4.** Western blot analysis of secreted protein from recombinant
Listeria encoding native or codon-optimized LLO secA2 signal peptide or codon-optimized
Tat signal peptide fused with codon-optimized EphA2 CO domain sequence.

[00138]   **Figure 5.** Flow cytometry analysis of human EphA2 expression in CT2 murine carcinoma cells. Single cell FACS sorting assays were performed by standard techniques to identify CT26 cell clones expressing high levels of human EphA2.

[00139]   **Figure 6.** Western blot analysis of pooled populations CT26 murine colon carcinoma cells expressing high levels of human EphA2 protein.

[00140]   **Figure 7.** Flow Cytometry of B16F10 cells expressing huEphA2.

[00141]   **Figure 8.** Western blot analysis of lysate from 293 cells 48 hr. following transfection with pCDNA4 plasmid DNA encoding full-length native EphA2 sequence.

[00142]   **Figures 9A-9B.** In the CT26 tumor model, therapeutic immunizaiton with positive control *Listeria* expressing AH1-A5.

[00143]   **Figures 10A-10B.** Preventative immunization with *Listeria* expressing ECD of hEphA2 suppresses CT26-hEphA2 tumor growth (Figure 10A) and increases survival (Figure 10B).

[00144]   **Figures 11A-11D.** Preventive studies following i.v. administration of L4029EphA2-exFlag, *Listeria* control (L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) (5×10^5 cells in 100 μl volume) either subcutaneously or intravenously. **Figure 11A** demonstrates tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2, vehicle (HBSS), *Listeria* (L4029) or *Listeria* positive (L4029-AH1) controls. **Figure 11B** demonstrates mean tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) compared to the *Listeria* (L4029) control. **Figure 11C** illustrates results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. **Figure 11D** illustrates the results of the prevention study in the lung metastases model, measuring percent survival of the mice post tumor cell inoculation.

[00145]   **Figure 12.** Preventative immunization with Listeria expressing ECD of hEphA2 increases survival following RenCa-hEphA2 tumor challenge.

[00146]   **Figures 13A-13C.** Figures 13A-13C illustrate results of a typical therapeutic study of animals inoculated with CT26 murine colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), Listeria control (L4029-control) or vehicle (HBSS). In **Figure 13A**, tumor volume was measured at several intervals post inoculation. **Figure 13B** illustrates the mean tumor volume of mice inoculated with CT26 cells containing either Listeria control or the ECD of huEphA2. **Figure 13C** represents the results of a therapeutic study using the lung metastases model, measuring percent survival of mice post inoculation with CT26 cells with either HBSS or Listeria control, or Listeria expressing the ECD of huEphA2.

[00147]   **Figures 14A-F. Figure 14A.** Therapeutic immunization in Balb/C mice with Listeria expressing ICD of hEphA2 suppresses established CT26-hEphA2 tumor growth. **Figure 14B.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant Listeria encoding EphA2 CO domain confers long-term survival. **Figure 14C.** Long-term survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors immunized with recombinant Listeria encoding OVA.AH1 or OVA.AH1-A5. **Figure 14D.** Increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors when immunized with recombinant Listeria encoding codon-optimized or native EphA2 CO domain sequence. **Figure 14E.** Increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors when immunized with recombinant Listeria encoding codon-optimized secA1 signal peptide fused with codon-optimized EphA2 EX2 domain sequence. **Figure 14F.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant Listeria encoding EphA2 CO domain but not with plasmid DNA encoding full-length EphA2 confers long-term survival.

[00148]   **Figure 15.** Long-term suppression of CT26-hEphA2 tumor growth upon rechallenge.

[00149]   **Figure 16.** Immunization with Listeria expressing hEphA2 elicits a specific CD8+ T cell response.

[00150]   **Figure 17.** Both CD4+ and CD8+ T cell responses are required for optimal hEphA2-directed anti-tumor efficacy.

[00151]   **Figures 18A-B.** Therapeutic vaccination with Listeria expressing human EphA2 ICD enhances CD45+ tumor infiltrate. **Figure 18A** depicts images of tumor sections stained with biotinylated rat anti-mouse CD45/B200. **Figure 18B** is a bar graph normalizing the image data to tumor volume.

5. DETAILED DESCRIPTION OF THE INVENTION

[00152]   The present invention is based, in part, on the inventors' discovery that a vaccine that comprises an EphA2 antigenic peptide can confer beneficial immune response against EphA2-expressing cells in involved in a hyperproliferative disease such as cancer. In particular, such a vaccine can contain an EphA2 antigenic peptide, an expression vehicle for an EphA2 antigenic peptide, or an antigen-presenting cell that is sensitized with an EphA2 antigenic peptide.

[00153]   The present invention provides methods and compositions that provide for the prevention, treatment, inhibition, and management of disorders associated with overexpression of EphA2, disorders associated with aberrant angiogenesis and/or hyperproliferative cell disorders. A particular aspect of the invention relates to methods and

compositions containing compounds that, when administered to a subject with a hyperproliferative cell disorder involving EphA2-expressing cells, either elicit or mediate an immune response against EphA2, resulting in a growth inhibition of the EphA2-expressing cells involved in the hyperproliferative cell disorder. The present invention

5   further relates to methods and compositions for the treatment, inhibition, or management of metastases of cancers of epithelial cell origin, especially human cancers of the breast, ovary, oesophagus, lung, skin, prostate, bladder, and pancreas, and renal cell carcinomas and melanomas. The invention further relates to methods and compositions for the prevention, treatment, inhibition, or management of cancers of T cell origin, especially leukemias and

10   lymphomas. Further, the compositions and methods of the invention include other types of active ingredients in combination with the EphA2 vaccines of the invention. In certain embodiments, the methods of the invention are used to treat, prevent or manage other non-neoplastic hyperproliferative cell disorders, for example, but not limited to asthma, psoriasis, restenosis, COPD, *etc.*

15   [00154]   The present invention also relates to methods for the treatment, inhibition, and management of cancer and hyperproliferative cell disorders that have become partially or completely refractory to current or standard therapy (*e.g.*, a cancer therapy, such as chemotherapy, radiation therapy, hormonal therapy, and biological-/immuno-therapy).

5.1 EphA2 Antigenic Peptides

20   [00155]   As discussed above, the invention encompasses administration of exogenous EphA2 antigenic peptides that are capable of eliciting an immune response to EphA2, resulting in a cellular or humoral immune response against endogenous EphA2. Additionally, the present invention encompasses the use of an EphA2 antigenic peptide expression vehicle.

25   [00156]   In principle, an EphA2 antigenic peptide (sometimes referred to as an "EphA2 antigenic polypeptide") for use in the methods and compositions of the present invention can be any EphA2 antigenic peptide that is capable of eliciting an immune response against EphA2-expressing cells involved in a hyperproliferative disorder. Thus, an EphA2 antigenic peptide can be an EphA2 polypeptide, preferably an EphA2

30   polypeptide of SEQ ID NO:2, or a fragment, analog or derivative of an EphA2 polypeptide that (1) displays antigenicity of EphA2 (ability to bind or compete with EphA2 for binding to an anti-EphA2 antibody, (2) displays immunogenicity of EphA2 (ability to generate antibody which binds to EphA2), and/or (3) contains one or more T cell epitopes of EphA2.

[00157]   In certain embodiments, the EphA2 antigenic peptide is a sequence encoded

35   by the nucleotide sequence provided below or a fragment or derivative thereof:

[00158]   Genbank Accession No. NM_004431   Human

[00159]   Genbank Accession No. NM_010139   Mouse

[00160]   Genbank Accession No. AB038986   Chicken (partial)

[00161]   In certain embodiments, the EphA2 antigenic peptide is full length human
EphA2 (SEQ ID NO:2).

[00162]   In other embodiments, the EphA2 antigenic peptide comprises the
intracellular domain of EphA2 (residue 22 to 554 of SEQ ID NO:2).

[00163]   In yet other embodiments, the EphA2 antigenic peptide comprises the
intracellular domain EphA2 (residue 558 to 976 of SEQ ID NO:2).

[00164]   In yet other embodiments, the EphA2 antigenic peptide comprises more than
one domain of the full length human EphA2. In a specific embodiment, the EphA2
antigenic peptides comprises the extracellular domain and the intracellular cytoplasmic
domain, joined together. In accordance with this embodiment, the transmembrane domain
of EphA2 is deleted.

[00165]   In certain embodiments of the invention, the tyrosine kinase activity of
EphA2 is ablated. Thus, EphA2 may contain deletions, additions or substitutions of amino
acid residues that result in the elimination of tyrosine kinase activity. In a preferred
embodiment, a lysine to methione substitution at position 646 is present.

[00166]   In a preferred embodiment, the EphA2 antigenic peptide comprises the
extracellular and cytoplasmic domains of EphA2 resulting from a deletion of the
transmembrane domain of EphA2 and has a lysine to methionine substitution as position
646.

[00167]   In certain embodiments, the peptide corresponds to or comprises an EphA2
epitope that is exposed in a cancer cell but occluded in a non-cancer cell. In a preferred
embodiment, the EphA2 antigenic peptides preferentially include epitopes on EphA2 that
are selectively exposed or increased on cancer cells but not non-cancer cells ("exposed
EphA2 epitope peptides").

[00168]   The present invention further encompasses the use of a plurality of EphA2
antigenic peptides, e.g., 2, 3, 4, 5, 6, or more EphA2 antigenic peptides, in the compositions
and methods of the present invention. In certain embodiments, the plurality of EphA2
antigenic peptides are multimerized or polyvalent.

[00169]   Fragments of EphA2 that are useful in the methods and compositions present
invention may contain deletions, additions or substitutions of amino acid residues within the
amino acid sequence encoded by an EphA2 gene. Preferably mutations result in a silent
change, thus producing a functionally equivalent EphA2 gene product. By "functionally

equivalent", it is meant that the mutated EphA2 gene product has the same function as the wild-type EphA2 gene product, e.g., contains one or more epitopes of EphA2.

[00170]   An EphA2 antigenic peptide sequence preferably comprises an amino acid sequence that exhibits at least about 65% sequence similarity to human EphA2, more

5   preferably exhibits at least 70% sequence similarity to human EphA2, yet more preferably exhibits at least about 75% sequence similarity human EphA2. In other embodiments, the EphA2 polypeptide sequence preferably comprises an amino acid sequence that exhibits at least 85% sequence similarity to human EphA2, yet more preferably exhibits at least 90% sequence similarity to human EphA2, and most preferably exhibits at least about 95%

10   sequence similarity to human EphA2.

[00171]   Additional polypeptides suitable in the present methods are those encoded by the nucleic acids described in Section 5.2 below.

[00172]   The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a

15   mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc Natl Acad Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc Natl Acad Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*,1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST

20   program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997,

25   *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[00173]   Another preferred, non limiting example of a mathematical algorithm

30   utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *Comput Appl Biosci* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional

35   algorithms for sequence analysis are known in the art and include ADVANCE and ADAM

as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA

described in Pearson and Lipman,1988, Proc Natl Acad Sci USA 85:2444-8. Within

FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup =

2, similar regions in the two sequences being compared are found by looking at pairs of

5   aligned residues; if ktup = 1, single aligned amino acids are examined. ktup can be set to 2

or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not

specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters,

see http://bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2.

[00174]   The percent identity between two sequences can be determined using

10   techniques similar to those described above, with or without allowing gaps. In calculating

percent identity, only exact matches are counted. However, conservative substitutions

should be considered in evaluating sequences that have a low percent identity with the

EphA2 sequences disclosed herein.

[00175]   In a specific embodiment, EphA2 antigenic peptides comprising at least 10,

15   20, 30, 40, 50, 75, 100, or 200 amino acids of an EphA2 polypeptide, preferably of SEQ ID

NO:2 are used in the present invention. In a preferred embodiment, EphA2 antigenic

peptides comprising at least 10, 20, 30, 40, 50, 75, 100, or 200 continguous amino acids of

an EphA2 polypeptide, preferably of SEQ ID NO:2 are used in the present invention. In a

preferred embodiment, such a polypeptide comprises all or a portion of the extracellular

20   domain of an EphA2 polypeptide of SEQ ID NO:2.

5.2   Methods of Identifying EphA2 Antigenic Peptides

5.2.1   Assays for EphA2 Antigenic Peptides

[00176]   The present invention provides *Listeria*-based EphA2 vaccines comprising

Listeria bacteria engineered to express an EphA2 antigenic peptide. Any assay known in

25   the art for determining whether a peptide is a T cell epitope or a B cell epitope may be

employed in testing EphA2 peptides for suitability in the present methods and

compositions.

[00177]   For example, for determining whether a peptide is a T cell epitope,

ELISPOT assays and methods for intracellular cytokine staining can be used for

30   enumeration and characterization of antigen-specific CD4^{+} and CD8^{+} T cells. Lalvani *et al.*

(1997) *J. Exp. Med.* 186:859-865; Waldrop *et al.* (1997) *J. Clin Invest.* 99:1739-1750.

[00178]   EphA2 antigenic peptides can be determined by screening synthetic peptides

corresponding to portions of EphA2. Candidate antigenic peptides can be identified on the

basis of their sequence or predicted structure. A number of algorithms are available for this purpose.

[00179]   Exemplary protocols for such assays are presented below.

5.2.2   Peptides That Display Immunogenicity of EphA2

5   [00180]   The ability of EphA2 peptides to elicit EphA2-specific antibody responses in mammals can be examined, for example, by immunizing animals (*e.g.*, mice, guinea pigs or rabbits) with individual EphA2 peptides emulsified in Freund's adjuvant.

[00181]   After three injections (5 to 100 μg peptide per injection), IgG antibody responses are tested by peptide-specific ELISAs and immunoblotting against EphA2.

10   [00182]   EphA2 peptides which produce antisera that react specifically with the EphA2 peptides and also recognized full length EphA2 protein in immunoblots are said to display the antigenicity of EphA2.

5.2.3   CD4$^+$ T-Cell Proliferation Assay

[00183]   For example, such assays include *in vitro* cell culture assays in which
15   peripheral blood mononuclear cells ("PBMCs") are obtained from fresh blood of a patient with a disease involving overexpression of EphA2, and purified by centrifugation using FICOLL-PLAQUE PLUS (Pharmacia, Upsalla, Sweden) essentially as described by Kruse and Sebald, 1992, *EMBO J.* 11:3237-3244. The peripheral blood mononuclear cells are incubated for 7-10 days with candidate EphA2 antigenic peptides. Antigen presenting cells
20   may optionally be added to the culture 24 to 48 hours prior to the assay, in order to process and present the antigen. The cells are then harvested by centrifugation, and washed in RPMI 1640 media (GibcoBRL, Gaithersburg, MD). 5 x 10^4 activated T cells/well are in RPMI 1640 media containing 10% fetal bovine serum, 10 mM HEPES, ph 7.5, 2 mM L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulphate in 96 well plates
25   for 72 hrs at 37°C, pulsed with 1 μCi ^{3}H-thymidine (DuPont NEN, Boston, MA)/well for 6 hrs, harvested, and radioactivity measured in a TOPCOUNT scintillation counter (Packard Instrument Col., Meriden, CT).

5.2.4   Intracellular Cytokine Staining (ICS)

[00184]   Measurement of antigen-specific, intracellular cytokine responses of T cells
30   can be performed essentially as described by Waldrop *et al.*, 1997, *J. Clin. Invest.* 99:1739-1750; Openshaw *et al.*, 1995, *J. Exp. Med.* 182:1357-1367; or Estcourt *et al.*, 1997, *Clin. Immunol. Immunopathol.* 83:60-67. Purified PBMCs from patients with a disease involving EphA2-overexpressing cells are placed in 12x75 millimeter polystyrene tissue culture tubes (Becton Dickinson, Lincoln Park, N.J.) at a concentration of 1x10^6 cells per tube. A

solution comprising 0.5 milliliters of HL-1 serum free medium, 100 units per milliliter of

penicillin, 100 units per milliliter streptomycin, 2 millimolar L glutamine (Gibco BRL),

varying amounts of individual EphA2 antigenic candidate peptides, and 1 unit of anti-CD28

mAb (Becton-Dickinson, Lincoln Park, N.J.) is added to each tube. Anti-CD3 mAb is

5   added to a duplicate set of normal PBMC cultures as positive control. Culture tubes are

incubated for 1 hour. Brefeldin A is added to individual tubes at a concentration of 1

microgram per milliliter, and the tubes are incubated for an additional 17 hours.

[00185]   PBMCs stimulated as described above are harvested by washing the cells

twice with a solution comprising Dulbecco's phosphate-buffered saline (dPBS) and 10 units

10 of Brefeldin A. These washed cells are fixed by incubation for 10 minutes in a solution

comprising 0.5 milliliters of 4% paraformaldehyde and dPBS. The cells are washed with a

solution comprising dPBS and 2% fetal calf serum (FCS). The cells are then either used

immediately for intracellular cytokine and surface marker staining or are frozen for no more

than three days in freezing medium, as described (Waldrop et al., 1997, J. Clin. Invest.

15 99:1739-1750).

[00186]   The cell preparations were rapidly thawed in a 37°C water bath and washed

once with dPBS. Cells, either fresh or frozen, are resuspended in 0.5 milliliters of

permeabilizing solution (Becton Dickinson Immunocytometry systems, San Jose, Calif.)

and incubated for 10 minutes at room temperature with protection from light.

20 Permeabilized cells are washed twice with dPBS and incubated with directly conjugated

mAbs for 20 minutes at room temperature with protection from light. Optimal

concentrations of antibodies are predetermined according to standard methods. After

staining, the cells were washed, refixed by incubation in a solution comprising dPBS 1%

paraformaldehyde, and stored away from light at 4°C for flow cytometry analysis.

25 ## 5.2.5   ELISPOT Assays

[00187]   The ELISPOT assay measures Th1-cytokine specific induction in murine

splenocytes following Listeria vaccination. ELISPOT assays are performed to determine

the frequency of T lymphocytes in response to endogenous antigenic peptide stimulation,

and are as described in Geginat, et al., 2001, J. Immunol. 166:1877-1884. Balb/c mice (3

30 per group) are vaccinated with L. monocytogenes expressing candidate EphA2 antigenic

peptides or HBSS as control. Whole mouse spleens are harvested and pooled five days

after vaccination. Single cell suspensions of murine splenocytes are plated in the presence

of various antigens overnight in a 37°C incubator.

[00188]   Assays are performed in nitrocellulose-backed 96-well microtiter plates

35 coated with rat anti-mouse IFN- γ mAb. For the testing of the candidate EphA2 antigenic

peptide, a 1 x 10⁻⁵ M peptide solution is prepared. In round-bottom 96-well microtiter

plates per well 6 x 10⁵ unseparated splenocytes in 135 µl culture medium (α modification of

Eagle's medium (Life Technologies, Eggenstein, Germany) supplemented with 10% FCS,

100 U/ml penicillin, 100 µg/ml streptomycin, 1 x 10⁻⁵ M 2-ME, and 2 mM glutamine) are

5   mixed with 15 µl of the 1 x 10⁻⁵ M peptide solution to yield a final peptide concentration of

1 x 10⁻⁶ M. After 6 h of incubation at 37°C, cells are resuspended by vigorous pipetting,

and 100 µl or 10 µl of cell suspension (4 x 10⁵/well or 4 x 10⁴/well, respectively) is

transferred to Ab-coated ELISPOT plates and incubated overnight at 37°C. In the

ELISPOT plates, the final volume was adjusted to 150 µl to ensure homogenous

10   distribution of cells.

[00189]   Purified CD4⁺ or CD8⁺ T cells are tested in a modified assay as follows: 15

µl prediluted peptide (1 x 10⁻⁵ M) is directly added to Ab-coated ELISPOT plates and

mixed with 4 x 10⁵ splenocytes from nonimmune animals as APC to yield a final volume of

100 µl. After 4 h of preincubation of APC at 37°C, 1 x 10⁵ CD4⁺ or CD8⁺ cells purified

15   from *L. monocytogenes*-immune mice are added per well in a volume of 50 µl and plates are

incubated overnight at 37°C. The ELISPOT-based *ex vivo* MHC restriction analysis is

performed after loading of cell lines expressing specific MHC class I molecules with 1 x 10⁻

⁶ M peptide for 2 h at 37°C. Subsequently, unbound peptides are washed off (four times) to

prevent binding of peptides to responder splenocytes. Per well of the ELISPOT plate, 1 x

20   10⁵ peptide-loaded APC are mixed with 4 x 10⁵ or 4 x 10⁴ responder splenocytes in a final

volume of 150 µl. After overnight incubation at 37°C, ELISPOT plates are developed with

biotin-labeled rat anti-mouse IFN- γ mAb, HRP streptavidin conjugate, and

aminoethylcarbazole dye of spots per splenocytes seeded. The specificity and sensitivity of

the ELISPOT assay is controlled with IFN- γ secreting CD8 T cell lines specific for a

25   control antigen.

5.3 Fusion Proteins

[00190]   In certain embodiments of the present invention, an EphA2 vaccine

comprises, expresses or is an antigen-presenting cell that is sensitized with an EphA2

antigenic peptide that is a fusion protein. Thus, the present invention encompasses

30   compositions and methods in which the EphA2 antigenic peptides are fusion proteins

comprising all or a fragment or derivative of EphA2 operatively associated to a

heterologous component, *e.g.*, a heterologous peptide. Heterologous components can

include, but are not limited to sequences which facilitate isolation and purification of the

fusion protein. Heterologous components can also include sequences which confer stability

to EphA2 antigenic peptides. Such fusion partners are well known to those of skill in the art.

[00191]   The present invention encompasses the use of fusion proteins comprising an EphA2 polypeptide (e.g., a polypeptide of SEQ ID NO:2 or a fragment thereof) and a

5   heterologous polypeptide (i.e., a polypeptide or fragment thereof, preferably a fragment of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 contiguous amino acids of the polypeptide). The fusion can be direct, but may occur through linker sequences. The heterologous polypeptide may be fused to the N-terminus or C-terminus of the EphA2 antigenic peptide. Alternatively, the

10   heterologous polypeptide may be flanked by EphA2 polypeptide sequences.

[00192]   A fusion protein can comprise an EphA2 antigenic peptide fused to a heterologous signal sequence at its N-terminus. Various signal sequences are commercially available. Eukaryotic heterologous signal sequences include, but are not limited to, the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene, La

15   Jolla, CA). Prokaryotic heterologous signal sequences useful in the methods of the invention include, but are not limited to, the phoA secretory signal (Sambrook *et al.*, eds., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and the protein A secretory signal (Pharmacia Biotech, Piscataway, NJ).

20   [00193]   The EphA2 antigenic peptide can be fused to tag sequences, e.g., a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available for use in the methods of the invention. As described in Gentz *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other

25   examples of peptide tags are the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell*, 37:767) and the "flag" tag (Knappik *et al.*, 1994, *Biotechniques*, 17(4):754-761). These tags are especially useful for purification of recombinantly produced EphA2 antigenic peptides.

[00194]   Any fusion protein may be readily purified by utilizing an antibody specific

30   or selective for the fusion protein being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:8972). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting

35   of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are

loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

[00195]   An affinity label can also be fused at its amino terminal to the carboxyl terminal of the EphA2 antigenic peptide for use in the methods of the invention. The

5   precise site at which the fusion is made in the carboxyl terminal is not critical. The optimal site can be determined by routine experimentation. An affinity label can also be fused at its carboxyl terminal to the amino terminal of the EphA2 antigenic peptide for use in the methods and compositions of the invention.

[00196]   A variety of affinity labels known in the art may be used, such as, but not

10   limited to, the immunoglobulin constant regions (see also Petty, 1996, Metal-chelate affinity chromatography, in Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience), glutathione S-transferase (GST; Smith, 1993, Methods Mol. Cell Bio. 4:220-229), the E. coli maltose binding protein (Guan et al., 1987, Gene 67:21-30), and various cellulose binding domains (U.S. Patent Nos. 5,496,934;

15   5,202,247; 5,137,819; Tomme et al., 1994, Protein Eng. 7:117-123), etc. Other affinity labels are recognized by specific binding partners and thus facilitate isolation by affinity binding to the binding partner which can be immobilized onto a solid support. Some affinity labels may afford the EphA2 antigenic peptide novel structural properties, such as the ability to form multimers. These affinity labels are usually derived from proteins that

20   normally exist as homopolymers. Affinity labels such as the extracellular domains of CD8 (Shiue et al., 1988, J. Exp. Med. 168:1993-2005), or CD28 (Lee et al., 1990, J. Immunol. 145:344-352), or fragments of the immunoglobulin molecule containing sites for interchain disulfide bonds, could lead to the formation of multimers.

[00197]   As will be appreciated by those skilled in the art, many methods can be used

25   to obtain the coding region of the above-mentioned affinity labels, including but not limited to, DNA cloning, DNA amplification, and synthetic methods. Some of the affinity labels and reagents for their detection and isolation are available commercially.

[00198]   In certain embodiments, the affinity label is a non-variable portion of the immunoglobulin molecule. Typically, such portions comprise at least a functionally

30   operative CH2 and CH3 domain of the constant region of an immunoglobulin heavy chain. Fusions are also made using the carboxyl terminus of the Fc portion of a constant domain, or a region immediately amino-terminal to the CH1 of the heavy or light chain. Suitable immunoglobulin-based affinity label may be obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD, or IgM, but preferably IgG1. Many DNA encoding immunoglobulin light or

35   heavy chain constant regions are known or readily available from cDNA libraries. See, for

example, Adams *et al.*, *Biochemistry*, 1980, 19:2711-2719; Gough *et al.*, 1980, *Biochemistry*, 19:2702-2710; Dolby *et al.*, 1980, *Proc. Natl. Acad. Sci. U.S.A.*, 77:6027-6031; Rice *et al.*, 1982, *Proc. Natl. Acad. Sci. U.S.A.*, 79:7862-7865; Falkner *et al.*, 1982, *Nature*, 298:286-288; and Morrison *et al.*, 1984, *Ann. Rev. Immunol*, 2:239-256. Because

5   many immunological reagents and labeling systems are available for the detection of immunoglobulins, the EphA2 antigenic peptide-Ig fusion protein can readily be detected and quantified by a variety of immunological techniques known in the art, such as the use of enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, fluorescence activated cell sorting (FACS), *etc.* Similarly, if the affinity label is an epitope with readily available

10   antibodies, such reagents can be used with the techniques mentioned above to detect, quantitate, and isolate the EphA2 antigenic peptide containing the affinity label. In many instances, there is no need to develop specific or selective antibodies to the EphA2 antigenic peptide for the purposes of purification.

      [00199]   A fusion protein can comprise an EphA2 antigenic peptide fused to the Fc

15   domain of an immunoglobulin molecule or a fragment thereof for use in the methods and compositions of the invention. A fusion protein can also comprise an EphA2 antigenic peptide fused to the CH2 and/or CH3 region of the Fc domain of an immunoglobulin molecule. Furthermore, a fusion protein can comprise an EphA2 antigenic peptide fused to the CH2, CH3, and hinge regions of the Fc domain of an immunoglobulin molecule (see

20   Bowen *et al.*, 1996, *J. Immunol.* 156:442-49). This hinge region contains three cysteine residues which are normally involved in disulfide bonding with other cysteines in the Ig molecule. Since none of the cysteines are required for the peptide to function as a tag, one or more of these cysteine residues may optionally be substituted by another amino acid residue, such as for example, serine.

25   [00200]   Various leader sequences known in the art can be used for the efficient secretion of the EphA2 antigenic peptide from bacterial and mammalian cells (von Heijne, 1985, J. *Mol. Biol.* 184:99-105). Leader peptides are selected based on the intended host cell, and may include bacterial, yeast, viral, animal, and mammalian sequences. For example, the herpes virus glycoprotein D leader peptide is suitable for use in a variety of

30   mammalian cells. A preferred leader peptide for use in mammalian cells can be obtained from the V-J2-C region of the mouse immunoglobulin kappa chain (Bernard *et al.*, 1981, *Proc. Natl. Acad. Sci.* 78:5812-5816). Preferred leader sequences for targeting EphA2 antigenic peptide expression in bacterial cells include, but are not limited to, the leader sequences of the *E.coli* proteins OmpA (Hobom *et al.*, 1995, *Dev. Biol. Stand.* 84:255-262),

35   Pho A (Oka *et al.*, 1985, *Proc. Natl. Acad. Sci* 82:7212-16), OmpT (Johnson *et al.*, 1996,

Protein Expression 7:104-113), LamB and OmpF (Hoffman & Wright, 1985, *Proc. Natl. Acad. Sci. USA* 82:5107-5111), β-lactamase (Kadonaga *et al.*, 1984, *J. Biol. Chem.* 259:2149-54), enterotoxins (Morioka-Fujimoto *et al.*, 1991, *J. Biol. Chem.* 266:1728-32), and the *Staphylococcus aureus* protein A (Abrahmsen *et al.*, 1986, *Nucleic Acids Res.* 14:7487-7500), and the *B. subtilis* endoglucanase (Lo *et al.*, *Appl. Environ. Microbiol.* 54:2287-2292), as well as artificial and synthetic signal sequences (MacIntyre *et al.*, 1990, *Mol. Gen. Genet.* 221:466-74; Kaiser *et al.*, 1987, *Science*, 235:312-317).

[00201]   In certain embodiments, the fusion partner comprises a non-EphA2 polypeptide corresponding to an antigen associated with the cell type against which a therapeutic or prophylactic immune is desired. For example, the non-EphA2 polypeptide can comprise an epitope of a tumor-associated antigen, such as, but not limited to, MAGE-1, MAGE-2, MAGE-3, gp100, TRP-2, tyrosinase, MART-1, β-HCG, CEA, Ras, β-catenin, gp43, GAGE-1, GAGE -2, N-acetylglucosaminyltransferase-V, p15, β-catenin, MUM-1, CDK4, HER-2/neu, Human papillomavirus-E6, Human papillomavirus-E7, and MUC-1, 2, 3.

[00202]   Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992).

[00203]   The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The expression of a fusion protein may be regulated by a constitutive, inducible or tissue-specific or -selective promoter. It will be understood by the skilled artisan that fusion proteins, which can facilitate solubility and/or expression, and can increase the *in vivo* half-life of the EphA2 antigenic peptide and thus are useful in the methods of the invention. The EphA2 antigenic peptides or peptide fragments thereof, or fusion proteins can be used in any assay that detects or measures EphA2 antigenic peptides or in the calibration and standardization of such assay.

[00204]   The methods of invention encompass the use of EphA2 antigenic peptides or peptide fragments thereof, which may be produced by recombinant DNA technology using

techniques well known in the art. Thus, methods for preparing the EphA2 antigenic

peptides of the invention by expressing nucleic acid containing EphA2 antigenic gene

sequences are described herein. Methods which are well known to those skilled in the art

can be used to construct expression vectors containing, e.g., EphA2 antigenic peptide

5   coding sequences (including but not limited to nucleic acids encoding all or an antigenic

portion of a polypeptide of SEQ ID NO:2) and appropriate transcriptional and translational

control signals. These methods include, for example, in vitro recombinant DNA

techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the

techniques described in Sambrook et al., 1989, supra, and Ausubel et al., 1989, supra.

10   Alternatively, RNA capable of encoding EphA2 antigenic peptide sequences may be

chemically synthesized using, for example, synthesizers (see, e.g., the techniques described

in Oligonucleotide Synthesis, 1984, Gait, M.J. ed., IRL Press, Oxford).

[00205]   In certain embodiments, the EphA2 antigenic peptide is functionally coupled

to an internalization signal peptide, also referred to as a "protein transduction domain," that

15   would allow its uptake into the cell nucleus. In certain specific embodiments, the

internalization signal is that of Antennapedia (reviewed by Prochiantz, 1996, Curr. Opin.

Neurobiol. 6:629 634, Hox A5 (Chatelin et al., 1996, Mech. Dev. 55:111 117), HIV TAT

protein (Vives et al., 1997, J. Biol. Chem. 272:16010 16017) or VP22 (Phelan et al., 1998,

Nat. Biotechnol. 16:440 443).

20   5.4 Polynucleotides Encoding An EphA2 Antigenic Peptide

[00206]   The present invention also encompasses compositions and methods that

employ an EphA2 antigenic peptide expression vehicle.

[00207]   In certain embodiments, the expression vehicles comprise or contain

polynucleotides that hybridize under high stringency, intermediate or lower stringency

25   hybridization conditions, e.g., as defined infra, to polynucleotides that encode an EphA2

antigenic peptide of the invention. In preferred embodiment, the expression vehicles

comprise or contain polynucleotides that hybridize, over their full length, under high

stringency, intermediate or lower stringency hybridization conditions, e.g., as defined infra,

to polynucleotides that encode an EphA2 antigenic peptide of the invention.

30   [00208]   By way of example and not limitation, procedures using such conditions of

low stringency for regions of hybridization of over 90 nucleotides are as follows (see also

Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792). Filters containing

DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC,

50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml

35   denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the

following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm
DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters
are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at
55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1%

5   SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at
60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are
washed for a third time at 65-68°C and re-exposed to film. Other conditions of low
stringency which may be used are well known in the art (e.g., as employed for cross-species
hybridizations).

10   [00209]   Also, by way of example and not limitation, procedures using such
conditions of high stringency for regions of hybridization of over 90 nucleotides are as
follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at
65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP,
0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are

15   hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/ml denatured
salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at
37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA.
This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography.

[00210]   Other conditions of high stringency which may be used depend on the nature

20   of the nucleic acid (e.g., length, GC content, etc.) and the purpose of the hybridization
(detection, amplification, etc.) and are well known in the art. For example, stringent
hybridization of a nucleic acid of approximately 15-40 bases to a complementary sequence
in the polymerase chain reaction (PCR) is done under the following conditions: a salt
concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a Mg^{2+}

25   concentration of 1.5 mM, a pH of 7-7.5 and an annealing temperature of 55-60°C.

[00211]   Selection of appropriate conditions for moderate stringencies is also well
known in the art (see, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory
Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; see
also, Ausubel et al., eds., in the Current Protocols in Molecular Biology series of laboratory

30   technique manuals, © 1987-1997, Current Protocols, © 1994-1997 John Wiley and Sons,
Inc.).

[00212]   The nucleic acids encoding EphA2 antigenic peptides useful in the present
methods may be made by any method known in the art. For example, if the nucleotide
sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may

35   be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier

et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the peptide, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

5   [00213]   Alternatively, a polynucleotide encoding an EphA2 antigenic peptide may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular peptide is not available, but the sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may be chemically synthesized or obtained from a suitable source (*e.g.*, a cDNA library, or a cDNA library generated from, or

10   nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing EphA2) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the peptide. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning

15   vectors using any method well known in the art.

[00214]   Further, a nucleic acid encoding an EphA2 antigenic peptide that is useful in the present methods may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, *etc.* (see, for example, the techniques described in Sambrook *et al.*,

20   1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate EphA2 antigenic peptides having a different amino acid sequence from the amino acid sequence depicted in SEQ ID NO:2, for example to create amino acid

25   substitutions, deletions, and/or insertions.

5.5 Recombinant Expression Of An EphA2 Antigenic Peptide

[00215]   Recombinant expression of an EphA2 antigenic peptide of the invention, or fragment or derivative thereof, requires construction of an expression vector containing a polynucleotide that encodes the EphA2 antigenic peptide. Once a polynucleotide encoding

30   an EphA2 antigenic peptide of the invention has been obtained, the vector for the production of the EphA2 antigenic peptide may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an EphA2 antigenic peptide-encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art

35   can be used to construct expression vectors containing peptide coding sequences and

appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an EphA2 antigenic peptide of the invention.

5   [00216]   The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an EphA2 antigenic peptide of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an EphA2 antigenic peptide of the invention or fragments thereof, operably linked to a heterologous promoter.

10   [00217]   A variety of host-expression vector systems may be utilized to express the EphA2 antigenic peptides of the invention (see, *e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an

15   EphA2 antigenic peptide of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing peptide coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing peptide coding sequences; insect cell systems infected with

20   recombinant virus expression vectors (*e.g.*, baculovirus) containing peptide coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing peptide coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells)

25   harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *E. coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant EphA2 antigenic peptide, are used for the expression of a recombinant

30   EphA2 antigenic peptide. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for peptides (Foecking *et al.*, 1986, *Gene* 45:101; and Cockett *et al.*, 1990, *BioTechnology* 8:2). In a specific embodiment, the expression of nucleotide sequences encoding an EphA2

antigenic peptide of the invention is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00218]   In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the EphA2 antigenic peptide being expressed.

5   For example, when a large quantity of such a protein is to be produced, for the generation of an EphA2 antigenic peptide vaccine, vectors which direct the expression of high levels of protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO* 12:1791), in which the peptide coding sequence may be ligated individually into the vector in frame

10   with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and

15   binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00219]   In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera*

20   *frugiperda* cells. The peptide coding sequence may be cloned individually into non-essential regions (*e.g.*, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter).

[00220]   In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the peptide coding

25   sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the EphA2 antigenic peptide in

30   infected hosts (*e.g.*, see Logan & Shenk, 1984, *PNAS* 8 1:355-359). Specific initiation signals may also be required for efficient translation of inserted peptide coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and

35   initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of

expression may be enhanced by the inclusion of appropriate transcription enhancer .

elements, transcription terminators, etc. (see, *e.g.*, Bittner *et al.*, 1987, *Methods in Enzymol.*

153:516-544).

[00221]   In addition, a host cell strain may be chosen which modulates the expression

5   of the inserted sequences, or modifies and processes the gene product in the specific fashion

desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein

products may be important for the function of the protein. Different host cells have

characteristic and specific mechanisms for the post-translational processing and

modification of proteins and gene products. Appropriate cell lines or host systems can be

10   chosen to ensure the correct modification and processing of the foreign protein expressed.

To this end, eukaryotic host cells which possess the cellular machinery for proper

processing of the primary transcript, glycosylation, and phosphorylation of the gene product

may be used. Such mammalian host cells include but are not limited to CHO, VERO,

BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O, NS1 and

15   T47D, NS0 (a murine myeloma cell line that does not endogenously produce any peptide

chains), CRL7O3O and HsS78Bst cells.

[00222]   For long-term, high-yield production of recombinant proteins, stable

expression is preferred. For example, cell lines which stably express the EphA2 antigenic

peptide may be engineered. Rather than using expression vectors which contain viral

20   origins of replication, host cells can be transformed with DNA controlled by appropriate

expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators,

polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the

foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media,

and then are switched to a selective media. The selectable marker in the recombinant

25   plasmid confers resistance to the selection and allows cells to stably integrate the plasmid

into their chromosomes and grow to form foci which in turn can be cloned and expanded

into cell lines. This method may advantageously be used to engineer cell lines which

express the EphA2 antigenic peptide. Such engineered cell lines may be particularly useful

in screening and evaluation of compositions that interact directly or indirectly with the

30   EphA2 antigenic peptide.

[00223]   A number of selection systems may be used, including but not limited to, the

herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, *Cell* 11:223), glutamine

synthetase, hypoxanthine guanine phosphoribosyltransferase (Szybalski & Szybalski, 1992,

Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy *et al.*,

35   1980, *Cell* 22:8-17) genes can be employed in tk-, gs-, hgprt- or aprt- cells, respectively.

Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, *PNAS* 77:357; O'Hare et al., 1981, *PNAS* 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *PNAS* 78:2072); neo, which confers resistance to the aminoglycoside G-418

5   (Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62: 191; May, 1993, *TIB TECH* 11:155-); and *hygro*, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant

10   clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1, which are incorporated by

15   reference herein in their entireties.

[00224]   The expression levels of an EphA2 antigenic peptide can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system

20   expressing peptide is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the peptide gene, production of the peptide will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

[00225]   Once an EphA2 antigenic peptide of the invention has been produced by

25   recombinant expression, it may be purified by any method known in the art for purification of an EphA2 antigenic peptide, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the EphA2 peptides of the present invention or

30   fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

5.6 Gene Therapy

[00226]   As discussed above, the present invention encompasses compositions and methods employ an EphA2 antigenic peptide expression vehicles. In certain embodiments,

35   the expression vehicle is any gene therapy vector available in the art can be used.

Exemplary gene therapy vectors that may be used as EphA2 antigenic peptide expression
vehicles are described below.

[00227]   For general reviews of the methods of gene therapy, *see*, Goldspiel *et al.*,
1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev,

5   1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932;
Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 1,
1(5):155-215. Methods commonly known in the art of recombinant DNA technology which
can be used are described in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology,
John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory

10   Manual, Stockton Press, NY (1990).

[00228]   In a preferred aspect, the expression vehicle comprises nucleic acid
sequences encoding an EphA2 antigenic peptide, said nucleic acid sequences being part of
expression vectors that express the EphA2 antigenic peptide in a suitable host. In
particular, such nucleic acid sequences have promoters operably linked to the EphA2

15   antigenic peptide, said promoter being inducible or constitutive, and, optionally, tissue-
specific. In another particular embodiment, nucleic acid molecules are used in which the
EphA2 antigenic peptide coding sequences and any other desired sequences are flanked by
regions that promote homologous recombination at a desired site in the genome, thus
providing for intrachromosomal expression of the EphA2 antigenic peptide (Koller and

20   Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature
342:435-438.

[00229]   Delivery of the nucleic acids into a subject may be either direct, in which
case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or
indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then

25   transplanted into the patient. These two approaches are known, respectively, as *in vivo* or
ex vivo gene therapy.

[00230]   In a specific embodiment, the nucleic acid sequences are directly
administered *in vivo*, where it is expressed to produce the encoded EphA2 antigenic
peptide. This can be accomplished by any of numerous methods known in the art, for

30   example by constructing them as part of an appropriate nucleic acid expression vector and
administering the vector so that the nucleic acid sequences become intracellular. Gene
therapy vectors can be administered by infection using defective or attenuated retrovirals or
other viral vectors (*see, e.g.*, U.S. Patent No. 4,980,286); direct injection of naked DNA;
use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont); coating with lipids

35   or cell-surface receptors or transfecting agents; encapsulation in liposomes, microparticles,

or microcapsules; administration in linkage to a peptide which is known to enter the

nucleus; administration in linkage to a ligand subject to receptor-mediated endocytosis (*see*,

e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell

types specifically expressing the receptors); *etc*. In another embodiment, nucleic acid-

5   ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to

disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet

another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and

expression, by targeting a specific receptor (*see*, *e.g.*, PCT Publications WO 92/06 180; WO

92/22635; WO92/20316; WO93/14188, and WO 93/20221). Alternatively, the nucleic acid

10   can be introduced intracellularly and incorporated within host cell DNA for expression by

homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA

86:8932-8935; Zijlstra *et al*., 1989, Nature 342:435-438).

[00231]   In a specific embodiment, viral vectors that contain nucleic acid sequences

encoding an EphA2 antigenic peptide. For example, a retroviral vector can be used (see

15   Miller *et al*., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors contain the

components necessary for the correct packaging of the viral genome and integration into the

host cell DNA. The nucleic acid sequences encoding the EphA2 antigenic peptide to be

used in gene therapy are cloned into one or more vectors, thereby facilitating delivery of the

gene into a patient. More detail about retroviral vectors can be found in Boesen *et al*., 1994,

20   Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr 1

gene to hematopoietic stem cells in order to make the stem cells more resistant to

chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy

are: Clowes *et al*., 1994, J. Clin. Invest. 93:644-651; Klein *et al*., 1994, Blood 83:1467-

1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and

25   Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

[00232]   Adenoviruses are other viral vectors that can be used in gene therapy.

Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia.

Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other

targets for adenovirus-based delivery systems are liver, the central nervous system,

30   endothelial cells, and muscle. Adenoviruses have the advantage of being capable of

infecting non-dividing cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and

Development 3:499-503 present a review of adenovirus-based gene therapy. Bout *et al*.,

1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer

genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of

35   adenoviruses in gene therapy can be found in Rosenfeld *et al*., 1991, Science 252:431-434;

Rosenfeld *et al.*, 1992, Cell 68:143-155; Mastrangeli *et al.*, 1993, J. Clin. Invest. 91:225-234; PCT Publication WO94/12649; and Wang *et al.*, 1995, Gene Therapy 2:775-783. In a preferred embodiment, adenovirus vectors are used.

[00233]   Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; and U.S. Patent No. 5,436,146).

[00234]   One approach to gene therapy encompassed by the present methods and compositions involves transferring a gene, *e.g.*, a nucleic acid encoding an EphA2 antigenic peptide, to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

[00235]   In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell mediated gene transfer, spheroplast fusion, *etc*. Numerous techniques are known in the art for the introduction of foreign genes into cells (*see, e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen *et al.*, 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[00236]   The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, *etc.*, and can be determined by one skilled in the art.

[00237]   Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to fibroblasts; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in

particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, *etc.*

[00238]   In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[00239]   In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding EphA2 antigenic peptide are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[00240]   In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.7 Expression Vehicles

[00241]   In certain embodiments, the present invention provides EphA2 antigenic peptide expression vehicles in the form of a microorganism, and, in specific embodiments, the microorganism is a bacterium.

[00242]   Microorganisms useful for the methods of the present invention include but are not limited to *Borrelia burgdorferi*, *Brucella melitensis*, *Escherichia coli*, enteroinvasive *Escherichia coli*, *Legionella pneumophila*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella spp.*, *Streptococcus spp.*, *Treponema pallidum*, *Yersinia enterocohtica*, *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeria seeligeri*, *Listeria welshimeri*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *BCG*, *Mycoplasma hominis*, *Rickettsiae quintana*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis carnii*, *Eimeria acervulina*, *Neospora caninum*, *Plasmodium falciparum*, *Sarcocystis suihominis*, *Toxoplasma gondii*, *Leishmania amazonensis*, *Leishmania major*, *Leishmania mexacana*, *Leptomonas karyophilus*, *Phytomonas spp.*, *Trypanasoma cruzi*, *Encephahtozoon cuniculi*, *Nosema helminthorum*, *Unikaryon legeri*. In certain specific embodiments, the bacteria is not *Listeria*, and more preferably is not *Listeria monocytogenes*. In certain embodiments, the bacteria is not *Bacillus anthracis*, *Cholera*, *Bordetalla pertussis*, *Corynebacterium*

diphtheriae, E. coli, Borrelia burgdorfer (Lyme), *Streptococcus pneumoniae, Salmonella, Staphylococcus sp., Mycobacterium tuberculosis, Brucella abortus, Brucella melitensis, Haemophilus influenzae, Neisseria meningitides, Yersinia pestis, Shigella sp., Francisella tulraensis, or Streptococcus pyogenes.*

5   [00243]   Another preferred infectious agent for use as an EphA2 antigenic peptide expression vehicle in accordance with the methods and compositions of the invention is a virus, for example, a DNA virus, including, but not limited to, an adenovirus, an adeno-associated virus, herpes simplex virus, or a RNA virus, including, but not limited to, a retrovirus, *e.g.*, a lentivirus. Preferred viruses for administration to human subjects are

10 attenuated viruses. A virus can be attenuated, for example, by exposing the virus to mutagens, such as ultraviolet irradiation or chemical mutagens, by multiple passages and/or passage in non-permissive hosts, and/or genetically altering the virus to reduce the virulence and pathogenicity of the virus. Representative patents disclosing attenuated viruses include U.S. Patent Nos. 6,689,367 (respiratory synctial virus), 6,326,007 (lentivirus), and

15 5,639,649 (rhinoviruses and enteroviruses).

[00244]   Many of the microorganisms encompassed by the present invention are causative agents of diseases in humans and animals. For example, sepsis from gram negative bacteria is a serious problem because of the high mortality rate associated with the onset of septic shock (R.C. Bone, 1993, Clinical Microbiol. Revs. 6:57-68). Therefore, to

20 allow the safe use of these microorganisms in both diagnostics and treatment of humans and animals, the microorganisms are attenuated in their virulence for causing disease. The end result is to reduce the risk of toxic shock or other side effects due to administration of the vector to the patient. In certain embodiments, the microorganisms have a decreased virulence (*e.g.*, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% less

25 virulent, or 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold less virulent) compared to the wild-type strain. In certain embodiments, the microorganisms have a decreased abililty to proliferate or replicate (*e.g.*, a 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% reduction in its ability to proliferate or replicate, or 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold reduction in its ability to

30 proliferate or replicate) compared to the wild-type strain. Examples of wild-type strains can be found in the American Type Culture Collection. Non-limiting examples of attenuated microoogranisms include antibiotic-sensitive strains of microorganisms, microorganism mutants that lack virulence factors, and strains of microorganisms with altered cell wall lipopolysaccharides.

[00245]   In certain embodiments, the attenuated microorganism is not naturally
occurring. In certain embodiments, the attenuated microorganism are isolated forms of
those that exist in nature. Attenuated microorganisms which are isolated forms of those that
exist in nature can be further attenuated and/or modified by the techniques known to those

5   of skill in the art.

[00246]   In certain embodiments, the microorganisms can be attenuated by the
deletion or disruption of DNA sequences which encode for virulence factors which insure
survival of the microorganisms in the host cell, especially macrophages and neutrophils, by,
for example, homologous recombination techniques and chemical or transposon

10   mutagenesis. Many, but not all, of these studied virulence factors are associated with
survival in macrophages such that these factors are specifically expressed within
macrophages due to stress, for example, acidification, or are used to induced specific host
cell responses, for example, macropinocytosis, Fields et al., 1986, Proc. Natl. Acad. Sci.
USA 83:5189-5193. Bacterial virulence factors include, for example: cytolysin; defensin

15   resistance loci; DNA K; fimbriae; GroEL; inv loci; lipoprotein; LPS; lysosomal fusion
inhibition; macrophage survival loci; oxidative stress response loci; pho loci (e.g., PhoP and
PhoQ); pho activated genes (pag; e.g., pagB and pagC); phoP and phoQ regulated genes
(prg); porins; serum resistance peptide; virulence plasmids (such as spvB, traT and ty2).

[00247]   Yet another method for the attenuation of the microorganisms is to modify

20   substituents of the microorganism which are responsible for the toxicity of that
microorganism. For example, lipopolysaccharide (LPS) or endotoxin is primarily
responsible for the pathological effects of bacterial sepsis. The component of LPS which
results in this response is lipid A (LA). Elimination or mitigation of the toxic effects of LA
results in an attenuated bacteria since 1) the risk of septic shock in the patient would be

25   reduced and 2) higher levels of the bacterial EphA2 antigenic peptide expression vehicle
could be tolerated.

[00248]   Rhodobacter (Rhodopseudomonas) sphaeroides and Rhodobacter capsulatus
each possess a monophosphoryl lipid A (MLA) which does not elicit a septic shock
response in experimental animals and, further, is an endotoxin antagonist. Loppnow et al.,

30   1990, Infect. Immun. 58:3743-3750; Takayma et al., 1989, Infect. Immun. 57:1336-1338.
Gram negative bacteria other than Rhodobacter can be genetically altered to produce MLA,
thereby reducing its potential of inducing septic shock.

[00249]   Yet another example for altering the LPS of bacteria involves the
introduction of mutations in the LPS biosynthetic pathway. Several enzymatic steps in LPS

35   biosynthesis and the genetic loci controlling them in a number of bacteria have been

identified, and several mutant bacterial strains have been isolated with genetic and enzymatic lesions in the LPS pathway. In certain embodiments, the LPS pathway mutant is a firA mutant. firA is the gene that encodes the enzyme UDP-3-O(R-30 hydroxymyristoyl)-glycocyamine N-acyltransferase, which regulates the third step in endotoxin biosynthesis (Kelley et al., 1993, J. Biol. Chem. 268:19866-19874).

[00250]   As a method of insuring the attenuated phenotype and to avoid reversion to the non-attenuated phenotype, the bacteria may be engineered such that it is attenuated in more than one manner, e.g., a mutation in the pathway for lipid A production and one or more mutations to auxotrophy for one or more nutrients or metabolites, such as uracil biosynthesis, purine biosynthesis, and arginine biosynthesis.

[00251]   In certain embodiments of the present invention, the bacterial EphA2 antigenic peptide expression vehicles are engineered to to be more susceptible to an antibiotic and/or to undergo cell death upon administration of a compound. For example, in one embodiment, the bacterial EphA2 antigenic peptide expression vehicles are engineered to deliver suicide genes to the target EphA2-expressing cells. These suicide genes include pro-drug converting enzymes, such as Herpes simplex thymidine kinase (TK) and bacterial cytosine deaminase (CD). TK phosphorylates the non-toxic substrates acyclovir and ganciclovir, rendering them toxic via their incorporation into genomic DNA. CD converts the non-toxic 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), which is toxic via its incorporation into RNA. Additional examples of pro-drug converting enzymes encompassed by the present invention include cytochrome p450 NADPH oxidoreductase which acts upon mitomycin C and porfiromycin (Murray et al., 1994, J. Pharmacol. Exp. Therapeut. 270:645-649). Other exemplary pro-drug converting enzymes that may be used in the methods and compositions of the present invention include: carboxypeptidase; beta-glucuronidase; penicillin-V-amidase; penicillin-G-amidase; beta-lactamase; beta.-glucosidase; nitroreductase; and carboxypeptidase A.

[00252]   Where the EphA2 vaccine comprises a microorganism that expresses an EphA2 antigenic peptide and, optionally, a pro-drug converting enzyme, the expression constructs are preferably designed such that the microorganism-produced peptides and enzymes are secreted by the microorganism. A number of bacterial secretion signals are well known in the art and may be used in the compositions and methods of the present invention. Exemplary secretion signals that can be used with gram-positive microorganisms include SecA (Sadaie et al., Gene 98:101-105, 1991), SecY (Suh et al., Mol. Microbiol. 4:305-314, 1990), SecE (Jeong et al., Mol. Microbiol. 10:133-142, 1993), FtsY an FfH (PCT/NL 96/00278), and PrsA (WO 94/19471). Exemplary secretion signals

that may be used with gram-negative microorganisms include those of soluble cytoplasmic proteins such as SecB and heat shock proteins; that of the peripheral membrane-associated protein SecA; and those of the integral membrane proteins SecY, SecE, SecD and SecF.

[00253]   The promoters driving the expression of the EphA2 antigenic peptides and, optionally, pro-drug converting enzymes, may be either constitutive, in which the peptides or enzymes are continually expressed, inducible, in which the peptides or enzymes are expressed only upon the presence of an inducer molecule(s), or cell-type specific control, in which the peptides or enzymes are expressed only in certain cell types. For example, a suitable inducible promoter can a promoter responsible for the baterial "SOS" response (Friedberg et al., In: DNA Repair and Mutagenesis, pp. 407-455, Am. Soc. Microbiol. Press, 1995). Such a promoter is inducible by numerous agents including chemotherapeutic alkylating agents such as mitomycin (Oda et al., 1985, Mutation Research 147:219-229; Nakamura et al., 1987, Mutation Res. 192:239-246; Shimda et al., 1994, Carcinogenesis 15:2523-2529) which is approved for use in humans. Promoter elements which belong to this group include umuC, sulA and others (Shinagawa et al., 1983, Gene 23:167-174; Schnarr et al., 1991, Biochemie 73:423-431). The sulA promoter includes the ATG of the sulA gene and the following 27 nucleotides as well as 70 nucleotides upstream of the ATG (Cole, 1983, Mol. Gen. Genet. 189:400-404). Therefore, it is useful both in expressing foreign genes and in creating gene fusions for sequences lacking initiating codons.

5.7.1   Exemplary Embodiment: *Listeria monocytogenes* As An Expression Vehicle

[00254]   *Listeria monocytogenes* (*Listeria*) is a Gram-positive facultative intracellular bacterium that is being developed for use in antigen-specific vaccines due to its ability to prime a potent CD4+/CD8+ T-cell mediated response via both MHC class I and class II antigen presentation pathways, and as such it has been tested recently as a vaccine vector in a human clinical trial among normal healthy volunteers.

[00255]   *Listeria* has been studied for many years as a model for stimulating both innate and adaptive T cell-dependent antibacterial immunity. The ability of *Listeria* to effectively stimulate cellular immunity is based on its intracellular lifecycle. Upon infecting the host, the bacterium is rapidly taken up by phagocytes including macrophages and dendritic cells into a phagolysosomal compartment. The majority of the bacteria are subsequently degraded. Peptides resulting from proteolytic degradation of pathogens within phagosomes of infected APCs are loaded directly onto MHC class II molecules, and these MHC II-peptide complexes activate CD4+ "helper" T cells that stimulate the production of antibodies, and the processed antigens are expressed on the surface of the

antigen presenting cell via the class II endosomal pathway. Within the acidic compartment, certain bacterial genes are activated including the cholesterol-dependent cytolysin, LLO, which can degrade the phagolysosome, releasing the bacterium into the cytosolic compartment of the host cell, where the surviving *Listeria* propagate. Efficient presentation

5   of heterologous antigens via the MHC class I pathway requires de novo endogenous protein expression by *Listeria*. Within antigen presenting cells (APC), proteins synthesized and secreted by *Listeria* are sampled and degraded by the proteosome. The resulting peptides are shuttled into the endoplasmic reticulum by TAP proteins and loaded onto MHC class I molecules. The MHC I-peptide complex is delivered to the cell surface, which in

10   combination with sufficient co-stimulation (signal 2) activates and stimulates cytotoxic T lymphocytes (CTLs) having the cognate T cell receptor to expand and subsequently recognize the MHC I-peptide complex.

[00256]   The EphA2 antigenic peptides are preferably expressed in *Listeria* using a heterologous gene expression cassette. A heterologous gene expression cassette is typically

15   comprised of the following ordered elements: (1) prokaryotic promoter; (2) Shine-Dalgarno sequence; (3) secretion signal (signal peptide); and, (4) heterologous gene. Optionally, the heterologous gene expression cassette may also contain a transcription termination sequence, in constructs for stable integration within the bacterial chromosome. While not required, inclusion of a transcription termination sequence as the final ordered element in a

20   heterologous gene expression cassette may prevent polar effects on the regulation of expression of adjacent genes, due to read-through transcription.

[00257]   The expression vectors introduced into the *Listeria*-based EphA2 vaccine are preferably designed such that the *Listeria*-produced EphA2 peptides and, optionally, a second tumor antigen, are secreted by the *Listeria*. A number of bacterial secretion signals

25   are well known in the art and may be used in the compositions and methods of the present invention. Exemplary secretion signals that can be used with gram-positive microorganisms include SecA (Sadaie *et al.*, 1991, *Gene* 98:101-105), SecY (Suh *et al.*, 1990, *Mol. Microbiol.* 4:305-314), SecE (Jeong *et al.*, 1993, *Mol. Microbiol.* 10:133-142), FtsY and FfH (PCT/NL 96/00278), and PrsA (WO 94/19471).

30   [00258]   The promoters driving the expression of the EphA2 antigenic peptides and, optionally, pro-drug converting enzymes, may be either constitutive, in which the peptides or enzymes are continually expressed, inducible, in which the peptides or enzymes are expressed only upon the presence of an inducer molecule(s), or cell-type specific, in which the peptides or enzymes are expressed only in certain cell types. For example, a suitable

35   inducible promoter can be a promoter responsible for the bacterial "SOS" response

(Friedberg *et al.*, In: DNA Repair and Mutagenesis, pp. 407-455, Am. Soc. Microbiol. Press, 1995). Such a promoter is inducible by numerous agents including chemotherapeutic alkylating agents such as mitomycin (Oda *et al.*, 1985, *Mutation Research* 147:219-229; Nakamura *et al.*, 1987, *Mutation Res.* 192:239-246; Shimda *et al.*, 1994, *Carcinogenesis*

5   15:2523-2529) which is approved for use in humans. Promoter elements which belong to this group include umuC, sulA and others (Shinagawa *et al.*, 1983, *Gene* 23:167-174; Schnarr *et al.*, 1991, *Biochemie* 73:423-431). The sulA promoter includes the ATG of the sulA gene and the following 27 nucleotides as well as 70 nucleotides upstream of the ATG (Cole, 1983, *Mol. Gen. Genet.* 189:400-404). Therefore, it is useful both in expressing

10   foreign genes and in creating gene fusions for sequences lacking initiating codons.

[00259]   Preferred embodiments of components of the EphA2 antigenic peptide expression system, to be used in conjunction with nucleic acids encoding EphA2 antigenic peptides described in Section 5.2, are provided below.

5.7.1.1. Construct Backbone

15   [00260]   One of ordinary skill in the art will recognize that a variety of plasmid construct backbones are available which are suitable for use in the assembly of a heterologous gene expression cassette. A particular plasmid construct backbone is selected based on whether expression of the heterologous gene expression cassette from the bacterial chromosome or from an extra-chromosomal episome is desired.

20   [00261]   Given as non-limiting examples, incorporation of the heterologous gene expression cassette into the bacterial chromosome of *Listeria monocytogenes* (*Listeria*) is accomplished with an integration vector that contains an expression cassette for a listeriophage integrase that catalyzes sequence-specific integration of the vector into the *Listeria* chromosome. For example, the integration vectors known as pPL1 and pPL2

25   program stable single-copy integration of a heterologous protein (e.g., EphA2-antigenic peptide) expression cassette within an innocuous region of the bacterial genome, and have been described in the literature (Lauer et. al., 2002, J. Bacteriol. 184:4177-4178). The integration vectors are stable as plasmids in E. coli and are introduced via conjugation into the desired *Listeria* background. Each vector lacks a *Listeria*-specific origin of replication

30   and encodes a phage integrase, such that the vectors are stable only upon integration into a chromosomal phage attachment site. Starting with a desired plasmid construct, the process of generating a recombinant *Listeria* strain expressing a desired protein(s) takes approximately one week. The pPL1 and pPL2 integration vectors are based, respectively, on the U153 and PSA listeriophages. The pPL1 vector integrates within the open reading

35   frame of the comK gene, while pPL2 integrates within the tRNAArg gene in such a manner

that the native sequence of the gene is restored upon successful integration, thus keeping its native expressed function intact. The pPL1 and pPL2 integration vectors contain a multiple cloning site sequence in order to facilitate construction of plasmids containing the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette. Alternatively,

5   incorporation of the EphA2-antigenic peptide expression cassette into the *Listeria* chromosome can be accomplished through alleleic exchange methods, known to those skilled in the art. In particular, compositions in which it is desired to not incorporate a gene encoding an antibiotic resistance protein as part of the construct containing the heterologous gene expression cassette, methods of allelic exchange are desirable. For example, the

10 pKSV7 vector (Camilli et. al. Mol. Microbiol. 1993 8,143-157), contains a temperature-sensitive *Listeria* Gram-positive replication origin which is exploited to select for recombinant clones at the non-permissive temperature that represent the pKSV7 plasmid recombined into the *Listeria* chromosome. The pKSV7 allelic exchange plasmid vector contains a multiple cloning site sequence in order to facilitate construction of plasmids

15 containing the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette, and also a chloramphenicol resistance gene. For insertion into the *Listeria* chromosome, the heterologous EphA2-antigenic peptide expression cassette construct is optimally flanked by approximately 1 kb of chromosomal DNA sequence that corresponds to the precise location of desired integration. The pKSV7-heterologous protein (e.g., EphA2-antigenic peptide)

20 expression cassette plasmid is introduced optimally into a desired bacterial strain by electroporation, according to standard methods for electroporation of Gram positive bacteria. Briefly, bacteria electroporated with the pKSV7-heterologous protein (e.g., EphA2-antigenic peptide) expression cassette plasmid are selected by plating on BHI agar media containing chloramphenicol (10 μg/ml), and incubated at the permissive temperature

25 of 30oC. Single cross-over integration into the bacterial chromosome is selected by passaging several individual colonies for multiple generations at the non-permissive temperature of 41oC in media containing chloramphenicol. Finally, plasmid excision and curing (double cross-over) is achieved by passaging several individual colonies for multiple generations at the permissive temperature of 30°C in BHI media not containing

30 chloramphenicol. Verification of integration of the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette into the bacteria chromosome can be accomplished by PCR, utilizing a primer pair that amplifies a region defined from within the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette to the bacterial chromosome targeting sequence not contained in the pKSV7 plasmid vector construct.

[00262]   In other compositions, it may be desired to express the heterologous protein
(e.g., EphA2-antigenic peptide) from a stable plasmid episome. Maintenance of the
plasmid episome through passaging for multiple generations requires the co-expression of a
protein that confers a selective advantage for the plasmid-containing bacterium. As non-

5   limiting examples, the protein co-expressed from the plasmid in combination with the
heterologous protein (e.g., EphA2-antigenic peptide) may be an antibiotic resistance
protein, for example chloramphenicol, or may be a bacterial protein (that is expressed from
the chromosome in wild-type bacteria), that can also confer a selective advantage. Non-
limiting examples of bacterial proteins include enzyme required for purine or amino acid

10   biosynthesis (selection under defined media lacking relevant amino acids or other necessary
precursor macromolecules), or a transcription factor required for the expression of genes
that confer a selective advantage in vitro or in vivo (Gunn et. al. 2001 J. Immuol. 167:6471-
6479). As a non-limiting example, pAM401 is a suitable plasmid for episomal expression
of a selected heterologous protein (e.g., EphA2-antigenic peptide) in diverse Gram-positive

15   bacterial genera (Wirth et. al. 1986 J. Bacteriol 165:831-836).

5.7.1.2. Shine-Dalgarno Sequence

[00263]   At the 3' end of the promoter is contained a poly-purine Shine-Dalgarno
sequence, the element required for engagement of the 30S ribosomal subunit (via 16S
rRNA) to the heterologous gene RNA transcript and initiation of translation. The Shine-

20   Dalgarno sequence has typically the following consensus sequence: (SEQ ID NO:66): 5'-
NAGGAGGU-N5-10-AUG (start codon)-3'. There are variations of the poly-purine Shine-
Dalgarno sequence Notably, the *Listeria* hly gene that encodes listerolysin O (LLO) has the
following Shine-Dalgarno sequence (SEQ ID NO:67): AAGGAGAGTGAAACCCATG
(Shine-Dalgarno sequence is underlined, and the translation start codon is bolded).

25   ### 5.7.1.3. Codon Optimization

[00264]   In some embodiments, for optimal translation efficiency of a selected
heterologous protein, it is desirable to utilize codons favored by Listeria. The preferred
codon usage for bacterial expression can be determined as described in Nakamura et al.,
2000, *Nucl. Acids Res.* 28:292. In some embodiments, codon-optimized expression of

30   EphA2 antigenic peptides, from *Listeria monocytogenes* is desired.

[00265]   The optimal codons utilized by *Listeria* monocytogenes for each amino acid
are shown in Table 3 below.

Table 3: *Listeria* Codon Bias: Codons to be used for optimizing expression

Amino Acid	One Letter Code	Optimal *Listeria* Codon

Alanine	A	GCA
Arginine	R	CGU
Asparagine	N	AAU
Aspartate	D	GAU
Cysteine	C	UGU
Glutamine	Q	CAA
Glutamate	E	GAA
Glycine	G	GGU
Histidine	H	CAU
Isoleucine	I	AUU
Leucine	L	UUA
Lysine	K	AAA
Methionine	M	AUG
Phenylalanine	F	UUU
Proline	P	CCA
Serine	S	AGU
Threonine	T	ACA
Tryptophan	W	UGG
Tyrosine	Y	UAU
Valine	V	GUU

5.7.1.4. Signal Peptides

[00266]   Bacteria utilize diverse pathways for protein secretion, including secA1 and Twin-Arg Translocation (Tat), which are located at the N-terminal end of the pre-protein. The majority of secreted proteins utilize the Sec pathway, in which the protein translocates through the bacterial membrane-embedded proteinaceous Sec pore in an unfolded conformation. In contrast, the proteins utilizing the Tat pathway are secreted in a folded conformation. Nucleotide sequence encoding signal peptides corresponding to either of these protein secretion pathways can be fused genetically in-frame to a desired heterologous protein coding sequence. The signal peptides optimally contain a signal peptidase at their carboxyl terminus for release of the authentic desired protein into the extra-cellular environment (Sharkov and Cai. 2002 J. Biol. Chem. 277:5796-5803; Nielsen et. al. 1997 Protein Engineering 10:1-6). Signal peptide cleavage sites can be predicted using programs such as SignalP 3.0 (Bendtsen et al., 2004, J. Mol. Biol. 340:783-795. The signal peptides can be derived not only from diverse secretion pathways, but also from diverse bacterial genera. Signal peptides have a common structural organization, having a charged N-terminus (N-domain), a hydrophobic core region (H-domain) and a more polar C-terminal region (C-domain), however, they do not show sequence conservation. The C-domain of the

signal peptide carries a type I signal peptidase (SPase I) cleavage site, having the consensus

sequence A-X-A, at positions −1 and −3 relative to the cleavage site. Proteins secreted via

the sec pathway have signal peptides that average 28 residues. Signal peptides related to

proteins secreted by the Tat pathway have a tripartite organization similar to Sec signal

5   peptides, but are characterized by having an RR-motif (R-R-X-#-#, where # is a

hydrophobic residue), located at the N-domain / H-domain boundary. Bacterial Tat signal

peptides average 14 amino acids longer than sec signal peptides. The Bacillus subtilis

secretome may contain as many as 69 putative proteins that utilize the Tat secretion

pathway, 14 of which contain a SPase I cleavage site (Jongbloed et. al. 2002 J. Biol. Chem.

10   277:44068-44078; Thalsma et. al., 2000 Microbiol. Mol. Biol. Rev. 64:515-547). Shown in

table 4 below are non-limiting examples of signal peptides that can be used in fusion

compositions with a selected heterologous gene, resulting in secretion from the bacterium of

the encoded protein.

[00267]   Table 4: signal sequences useful for bacterial expression and secretion of

15   EphA2.

Secretion Pathway	Signal Peptide Amino Acid Sequence (NH₂-CO₂)	Signal peptidase Site (cleavage site represented by ')	Gene	Genus/species	SEQ ID NO:
secA1	MKKIMLVFITLILVSLPI AQQTEAKD	TEA'KD (SEQ ID NO:70)	hly (LLO)	Listeria monocytogene s	44
	MTDKKSENQTEKTETK ENKGMTRREMLKLSAV AGTGIAVGATGLGTILN VVDQVDKALT	DKA'LT (SEQ ID NO:71)	lmo0367	Listeria monocytogene s	45
	MAYDSRFDEWVQKLK EESFQNNTFDRRKFIQG AGKIAGLSLGLTIAQSV GAFG	VGA'FG (SEQ ID NO:72)	PhoD (alkaline phosphata se)	Bacillus subtillis	46

[00268]   It should be appreciated by those skilled in the art that there exists a variety

of proteins secreted via the Tat pathway among diverse bacterial genera, and that selected

Tat signal peptides corresponding to these proteins can be fused genetically in-frame to a

desired sequence encoding a heterologous protein, to facilitate secretion of the functionally

20   linked Tat signal peptide-heterologous protein chimera via the Tat pathway. Provided

below are non-limiting examples of proteins from *Bacillus subtilis* and *Listeria* (*innocua*

and *monocytogenes*) that are predicted to utilize Tat pathway secretion.

Putative *Bacillus subtilis* Proteins Secreted by Tat
Pathwayhttp://www.sas.upenn.edu/~pohlschr/TABLE1.html

25   [00269]   >gi|2635523|emb|CAB15017.1| similar to two (component sensor histidine

kinase (YtsA) (Bacillus subtilis)

[00270]   >gi|2632548|emb|CAB12056.1| phosphodiesterase/alkaline phosphatase D (Bacillus subtilis)

[00271]   >gi|2632573|emb|CAB12081.1| similar to hypothetical proteins (Bacillus subtilis)

5   [00272]   >gi|2633776|emb|CAB13278.1| similar to hypothetical proteins (Bacillus subtilis)

[00273]   >gi|2634674|emb|CAB14172.1| menaquinol:cytochrome c oxidoreductase (iron (sulfur subunit) (Bacillus subtilis)

[00274]   >gi|2635595|emb|CAB15089.1| yubF (Bacillus subtilis)

10   [00275]   >gi|2636361|emb|CAB15852.1| alternate gene name: ipa (29d~similar to hypothetical proteins (Bacillus subtilis)

<div align="center">

Putative *Listeria* Proteins Secreted by Tat
Pathwayhttp://www.sas.upenn.edu/~pohlschr/TABLE1.html

</div>

[00276]   *Listeria innocua*http://www.sas.upenn.edu/~pohlschr/TABLE1.html

15   [00277]   >gi|16799463|ref|NP_469731.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* innocua)

[00278]   >gi|16801368|ref|NP_471636.1| similar to 3 (oxoacyl (acyl (carrier protein synthase (*Listeria* innocua)

[00279]   *Listeria monocytogenes EGD (e)*

20   http://www.sas.upenn.edu/~pohlschr/TABLE1.html

[00280]   . >gi|16802412|ref|NP_463897.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* monocytogenes EGD (e)

[00281]   Organisms utilize codon bias to regulate expression of particular endogenous genes. Thus, signal peptides utilized for secretion of selected heterologous proteins may not

25   contain codons that utilize preferred codons, resulting in non-optimal levels of protein synthesis. In some some embodiments, the signal peptide sequence fused in frame with a gene encoding a selected heterologous protein is codon-optimized for codon usage in a selected bacterium. In some embodiments for expression and secretion from recombinant *Listeria monocytogenes*, a nucleotide sequence of a selected signal peptide is codon

30   optimized for expression in *Listeria monocytogenes*, according to Table 4 *supra*.

<div align="center">

5.7.1.5. Transcription Termination Sequence

</div>

[00282]   In some embodiments, a transcription termination sequence can be inserted into the heterologous protein expression cassette, downstream from the C-terminus of the translational stop codon related to the heterologous protein. Appropriate sequence elements

35   known to those who are skilled in the art that promote either rho-dependent or rho-

<div align="center">69</div>

independent transcription termination can be placed in the heterologous protein expression
cassette.

5.8 Anti-Idiotypic Antibodies

[00283]   The present invention relates to methods and compositions utilizing EphA2
vaccines for eliciting immune responses against EphA2-expressing cells and treatment and
prevention of disorders involving EphA2-expressing cells. In certain embodiments, the
EphA2 vaccines of the invention comprises an anti-idiotype of an anti-EphA2 antibody.

[00284]   The idiotopes on a single antibody molecule are thought to mimic and be the
"internal image" of any foreign or self epitope at the molecular level. By means of Mab
technology, an antibody against an EphA2 epitope is produced, purified and subsequently
used as an immunogen to elicit an anti-idiotypic antibody which may be an internal image
of the original EphA2 epitope. Thus, as predicted by the Jerne "network" theory (Jerne,
1974, *Ann. Inst. Pasteur. Immun.* 125C:373-389), immunization with an anti-idiotypic
antibody that is directed against antigen combining sites of an anti-EphA2 epitope antibody
would elicit a humoral immune response specific for the nominal antigen. The resulting
anti-anti-idiotypic antibody should react with the original primary EphA2 epitope.

[00285]   Thus, EphA2 antibodies can be utilized to generate anti-idiotype antibodies
that using techniques well known to those skilled in the art. (*See, e.g.*, Greenspan & Bona,
1993, *FASEB* 17(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8)2429-2438). For
example, antibodies which bind to EphA2 can be used to generate anti-idiotypes that, when
administered to a subject, can elicit a humoral immune response against EphA2. Such anti-
idiotypes (including molecules comprising, or alternatively consisting of, antibody
fragments or variants, such as Fab fragments of such anti-idiotypes) can be used in
therapeutic regimens to elicit an immune response against hyperproliferative cells that
express EphA2 and thus be useful in treating, preventing or managing hyperproliferative
diseases involving EphA2-overexpressing cells.

5.9 Prophylactic/Therapeutic Methods

[00286]   The present invention provides methods for treating, preventing, or
managing a disorder associated with overexpression of EphA2 and/or hyperproliferative
cell disorders, preferably cancer, comprising administering to a subject in need thereof one
or more EphA2 vaccines of the invention.

[00287]   The present invention encompasses methods for eliciting an immune
response against an EphA2-expressing cell associated with a hyperproliferative cell
disorder, comprising administering to a subject one or more EphA2 vaccines of the

invention in an amount effective for eliciting an immune response against the EphA2-
expressing cell.

[00288] An EphA2 vaccine may comprise one more EphA2 antigenic peptides, one
ore more EphA2 antigenic peptide expression vehicles, or antigen presenting cells

5   sensitized with one ore more EphA2 antigenic peptides.

[00289] In another specific embodiment, the disorder to be treated, prevented, or
managed is a pre-cancerous condition associated with cells that overexpress EphA2. In
more specific embodiments, the pre-cancerous condition is high-grade prostatic
intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or

10   compound nevi.

[00290] The present invention provides methods for treating, preventing, or
managing a disorder associated with overexpression of EphA2 and/or hyperproliferative
cell disorders, preferably cancer, comprising administering to a subject in need thereof one
or more EphA2 vaccines of the invention and one or more other therapies. Examples of

15   other therapies include, but are not limited to, those listed below in Section 5.9.3, *infra*. In
one embodiment, the peptides of the invention can be administered in combination with one
or more other therapies (e.g., prophylactic or therapeutic agents) useful in the treatment,
prevention or management of disorders associated with EphA2 overexpression and/or
hyperproliferative cell disorders, such as cancer. In certain embodiments, one or more

20   EphA2 antigenic peptides of the invention are administered to a subject, preferably a
human, concurrently with one or more other therapies (*e.g.*, therapeutic agents) useful for
the treatment or management of cancer. The term "concurrently" is not limited to the
administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same
time, but rather it is meant that an EphA2 antigenic peptide of the invention and another

25   therapy are administered to a subject in a sequence and within a time interval such that the
EphA2 antigenic peptide of the invention can act together with the other therapy to provide
an increased benefit than if they were administered otherwise. For example, each therapy
(*e.g.*, prophylactic or therapeutic agent) may be administered at the same time or
sequentially in any order at different points in time; however, if not administered at the

30   same time, they should be administered sufficiently close in time so as to provide the
desired therapeutic or prophylactic effect. Each therapy (*e.g.*, prophylactic or therapeutic
agent) can be administered separately, in any appropriate form and by any suitable route. In
certain embodiments, the EphA2 antigenic peptides of the invention are administered
before, concurrently or after surgery. Preferably the surgery completely removes localized

tumors or reduces the size of large tumors. Surgery can also be done as a preventive measure or to relieve pain.

[00291]   In various embodiments, the therapies (e.g., prophylactic or therapeutic agents) are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In preferred embodiments, two or more therapies are administered within the same patient visit.

[00292]   The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56th ed., 2002, 57th ed., 2003, and 58th ed., 2004).

[00293]   In certain embodiments of the invention, methods of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells or a disorder associated with aberrant angiogenesis are provided, said method comprising (1) administering to the individual a composition comprising an EphA2 expression vehicle, selected from the group consisting of a bacterium or virus, in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells; and (2) administering to the individual an antibiotic or antiviral agent in an amount effective to treat a bacterial or viral infection. In certain embodiments, the antibiotic or antiviral agent is administered within a short period of time following administration of the EphA2 expression vehicle (e.g., 30 minutes, 1 hour, 2 hours, 4 hours, 5 hours, or 5-30 minutes, 30 minutes to 1 hours, 1-5 hours.

5.9.1   **Patient Population**

[00294]   The invention provides methods for treating, preventing, and/or managing a disorder associated with EphA2 overexpression and/or hyperproliferative cell disease, particularly cancer, comprising administrating to a subject in need thereof one or more

EphA2 vaccines of the invention in a therapeutically or prophylactically effective amount or
an amount effective to elicit an immune response against EphA2-expressing cells associated
with the hyperproliferative disorder. In another embodiment, an effective amount of an
EphA2 vaccine of the invention is administered in combination with an effective amount of

5   one or more other therapies (*e.g.*, therapeutic or prophylactic agents) to treat, prevent,
and/or manage a disorder associated with EphA2 overexpression and/or hyperproliferative
cell disease, particularly cancer. The subject is preferably a mammal such as non-primate
(*e.g.*, cows, pigs, horses, cats, dogs, rats, *etc.*) and a primate (*e.g.*, monkey, such as a
cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

10   [00295]   Specific examples of cancers that can be treated by the methods
encompassed by the invention include, but are not limited to, cancers that overexpress
EphA2. In one embodiment, the cancer is of an epithelial origin. Examples of such cancers
are cancer of the lung, colon, ovary, esophagus, prostate, breast, and skin. Other cancers
include cancer of the bladder and pancreas and renal cell carcinoma and melanoma. In

15   another embodiment, the cancer is a solid tumor. In another embodiment, the cancer is of a
T cell origin. Examples of such cancers are leukemias and lymphomas. Additional cancers
are listed by example and not by limitation in the following Section 5.9.1.1. In particular
embodiments, methods of the invention can be used to treat and/or prevent metastasis from
primary tumors.

20   [00296]   The methods and compositions of the invention comprise the administration
of one or more EphA2 vaccines of the invention to subjects/patients suffering from or
expected to suffer from cancer, *e.g.*, have a genetic predisposition for a particular type of
cancer, have been exposed to a carcinogen, or are in remission from a particular cancer. As
used herein, "cancer" refers to primary or metastatic cancers. Such patients may or may not

25   have been previously treated for cancer. The methods and compositions of the invention
may be used as any line of cancer therapy, e.g., a first line, second line, or third line of
cancer therapy. Included in the invention is also the treatment of patients undergoing other
cancer therapies and the methods and compositions of the invention can be used before any
adverse effects or intolerance of these other cancer therapies occurs. The invention also

30   encompasses methods for administering one or more EphA2 vaccines of the invention to
treat or ameliorate symptoms in refractory patients. In a certain embodiment, that a cancer
is refractory to a therapy means that at least some significant portion of the cancer cells are
not killed or their cell division arrested. The determination of whether the cancer cells are
refractory can be made either *in vivo* or *in vitro* by any method known in the art for

35   assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of

"refractory" in such a context. In various embodiments, a cancer is refractory where the number of cancer cells has not been significantly reduced, or has increased. The invention also encompasses alternative therapies for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof patients in which chemotherapy,

5   radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy has proven or may prove too toxic, i.e., results in unacceptable or unbearable side effects, for the patient being undergoing said therapy. The invention also encompasses methods for administering one or more EphA2 vaccines to prevent the onset or recurrence of cancer in patients predisposed to having cancer. The invention also encompasses methods for

10   preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients with mean absolute lymphocyte cell counts of at least 500 cells/ mm^3, preferably at least 600 cells/ mm^3, more preferably at least 750 cells/ mm^3.

[00297]   In particular embodiments, the EphA2 vaccines of the invention are administered to reverse resistance or reduced sensitivity of cancer cells to certain hormonal,

15   radiation and chemotherapeutic agents thereby resensitizing the cancer cells to one or more of these agents, which can then be administered (or continue to be administered) to treat or manage cancer, including to prevent metastasis. In a specific embodiment, the EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different

20   treatment regimens, such as chemotherapy and hormonal therapy. In another specific embodiment, the EphA2 vaccines of the invention are administered to patients suffering from breast cancer that have a decreased responsiveness or are refractory to tamoxifen treatment. In another specific embodiment, the EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been

25   associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy.

[00298]   In alternate embodiments, the invention provides methods for treating or managing a patients' cancer comprising administering to the patient one or more EphA2 vaccines of the invention in combination with any other therapy or to patients who have

30   proven refractory to other therapies but are no longer on these therapies. In certain embodiments, the patients being treated by the methods of the invention are patients already being treated with chemotherapy, radiation therapy, hormonal therapy, or biological therapy/immunotherapy. Among these patients are refractory patients and those with cancer despite treatment with existing cancer therapies. In other embodiments, the patients

have been treated and have no disease activity and one or more EphA2 vaccines of the invention are administered to prevent the recurrence of cancer.

[00299]   In preferred embodiments, the existing therapy is chemotherapy. In particular embodiments, the existing therapy includes administration of chemotherapies
5   including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc. Among these
10   patients are patients treated with radiation therapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment or management of cancer.

[00300]   Alternatively, the invention also encompasses methods for treating patients undergoing or having undergone radiation therapy. Among these are patients being treated
15   or previously treated with chemotherapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00301]   In other embodiments, the invention encompasses methods for treating patients undergoing or having undergone hormonal therapy and/or biological
20   therapy/immunotherapy. Among these are patients being treated or having been treated with chemotherapy and/or radiation therapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00302]   Additionally, the invention also provides methods of treatment or management of cancer as an alternative to chemotherapy, radiation therapy, hormonal
25   therapy, and/or biological therapy/immunotherapy where the therapy has proven or may prove too toxic, i.e., results in unacceptable or unbearable side effects, for the subject being treated. The subject being treated with the methods of the invention may, optionally, be treated with other cancer therapies such as surgery, chemotherapy, radiation therapy, hormonal therapy or biological therapy, depending on which therapy was found to be
30   unacceptable or unbearable.

[00303]   In other embodiments, the invention provides administration of one or more EphA2 vaccines of the invention without any other cancer therapies for the treatment of cancer, but who have proved refractory to such treatments. In specific embodiments, patients refractory to other cancer therapies are administered one or more EphA2 vaccines
35   in the absence of cancer therapies.

[00304]   In other embodiments, patients with a pre-cancerous condition associated with cells that overexpress EphA2 can be administered vaccines of the invention to treat the disorder and decrease the likelihood that it will progress to malignant cancer. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00305]   In yet other embodiments, the invention provides methods of treating, preventing and/or managing hyperproliferative cell disorders other than cancer, particularly those associated with overexpression of EphA2, including but not limited to, asthma, chromic obstructive pulmonary disorder (COPD), fibrosis (*e.g.*, lung, kidney, heart and liver fibrosis), restenosis (smooth muscle and/or endothelial), psoriasis, etc. These methods include methods analogous to those described above for treating, preventing and managing cancer, for example, by administering the EphA2 vaccines of the invention, combination therapy (see, *e.g.*, Section 5.9.3, *infra*, for examples of other therapies to administer in combination with the EphA2 vaccines to a subject to treat, prevent or manage a hyperproliferative disorder other than cancer), administration to patients refractory to particular treatments, etc.

5.9.1.1. Cancers

[00306]   Cancers and related disorders that can be treated, prevented, or managed by methods and compositions of the present invention include but are not limited to cancers of an epithelial cell origin and/or endothelial cell origin. Examples of such cancers include the following: leukemias, such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias, such as, myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia leukemias and myelodysplastic syndrome; chronic leukemias, such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited

to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial

tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma,

pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including but not

limited to ductal carcinoma, adenocarcinoma, lobular (small cell) carcinoma, intraductal

5   carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer,

papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer

such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer

such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer

and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma,

10   gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell

tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor,

acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma

such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and

retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and

15   melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma,

basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited

to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited

to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to,

ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor;

20   esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid

cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma,

melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma;

stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid),

ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma,

25   fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but

not limited to hepatocellular carcinoma and hepatoblastoma; gallbladder cancers such as

adenocarcinoma; cholangiocarcinomas such as but not limited to pappillary, nodular, and

diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma

(epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer;

30   testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic

(typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma,

choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, prostatic

intraepithelial neoplasia, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal

cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers;

35   salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid

carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney

5   cancers such as but not limited to renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma,

10   hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary . adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin

15   Books U.S.A., Inc., United States of America)

[00307]   Accordingly, the methods and compositions of the invention are also useful in the treatment or prevention of a variety of cancers or other abnormal proliferative diseases, including (but not limited to) the following: carcinoma, including that of the bladder, breast, ovary, oesophagus, colon, kidney, liver, lung, ovary, pancreas, stomach,

20   cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma;

25   other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular

30   cancer and teratocarcinoma. It is also contemplated that cancers caused by aberrations in apoptosis would also be treated by the methods and compositions of the invention. Such cancers may include but not be limited to follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. In

35   specific embodiments, malignancy or dysproliferative changes (such as metaplasias and

dysplasias), or hyperproliferative disorders, are treated or prevented in the skin, lung, colon, breast, prostate, bladder, kidney, pancreas, ovary, or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented.

[00308] In some embodiments, the cancer is malignant and overexpresses EphA2. In other embodiments, the disorder to be treated is a pre-cancerous condition associated with cells that overexpress EphA2. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00309] In preferred embodiments, the methods and compositions of the invention are used for the treatment and/or prevention of breast, colon, ovarian, oesophageal, lung, and prostate cancers and melanoma and are provided below by example rather than by limitation.

[00310] In another preferred embodiment, the methods and compositions of the invention are used for the treatment and/or prevention of cancers of T cell origin, including, but not limited to, leukemias and lymphomas.

[00311] In certain embodiments, the methods of the invention, when used to treat a patient, result in decreased tumor volume (e.g., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95%, or 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold less) compared to the tumor volume prior to treatment.

[00312] In other embodiments, the methods of the invention, when used to treat a patient, result in an increased survival time (e.g., by 5, 10, 15, 20, 30, 35, 40, 45, 50 weeks, or 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 years, or by 1-6 months, 6-12 months, 1-5 years, 5-10 years, 10-20 years, 20-30 years or more.

5.9.1.2. Treatment of Breast Cancer

[00313] In specific embodiments, patients with breast cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for breast cancer therapy including but not limited to: doxorubicin, epirubicin, the combination of doxorubicin and cyclophosphamide (AC), the combination of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF), the combination of cyclophosphamide, epirubicin and 5-fluorouracil (CEF), her-2 antibodies, e.g., herceptin, tamoxifen, the combination of tamoxifen and cytotoxic chemotherapy, taxanes (such as docetaxel and paclitaxel). In a further embodiment, peptides of the invention can be administered with taxanes plus standard doxorubicin and cyclophosphamide for adjuvant treatment of node-positive, localized breast cancer.

[00314]   In a specific embodiment, patients with pre-cancerous fibroadenoma of the breast or fibrocystic disease are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant breast cancer. In another specific embodiment, patients refractory to treatment, particularly hormonal

5   therapy, more particularly tamoxifen therapy, are administered an EphA2 vaccine of the invention to treat the cancer and/or render the patient non-refractory or responsive.

5.9.1.3. Treatment of Colon Cancer

[00315]   In specific embodiments, patients with colon cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment,

10   the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for colon cancer therapy including but not limited to: AVASTIN™ (bevacizumab), the combination of 5-FU and leucovorin, the combination of 5-FU and levamisole, irinotecan (CPT-11) or the combination of irinotecan, 5-FU and leucovorin (IFL).

15   ### 5.9.1.4. Treatment of Prostate Cancer

[00316]   In specific embodiments, patients with prostate cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for prostate cancer therapy including but not limited to:

20   external-beam radiation therapy, interstitial implantation of radioisotopes (i.e., I^{125}, palladium, iridium), leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), the combination of leuprolide and flutamide, estrogens such as DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate, radioisotopes, such as strontium-

25   89, the combination of external-beam radiation therapy and strontium-89, second-line hormonal therapies such as aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, and ketoconazole, low-dose prednisone, or other chemotherapy regimens reported to produce subjective improvement in symptoms and reduction in PSA level including docetaxel, paclitaxel, estramustine/docetaxel, estramustine/etoposide,

30   estramustine/vinblastine, and estramustine/paclitaxel.

[00317]   In a specific embodiment, patients with pre-cancerous high-grade prostatic intraepithelial neoplasia (PIN) are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant prostate cancer.

5.9.1.5. Treatment of Melanoma

[00318]   In specific embodiments, patients with melanoma are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for melanoma cancer therapy including but not limited

5   to: dacarbazine (DTIC), nitrosoureas such as carmustine (BCNU) and lomustine (CCNU), agents with modest single agent activity including vinca alkaloids, platinum compounds, and taxanes, the Dartmouth regimen (cisplatin, BCNU, and DTIC), interferon alpha (IFN-A), and interleukin-2 (IL-2). In a specific embodiment, an effective amount of one or more EphA2 vaccines of the invention can be administered in combination with isolated

10 hyperthermic limb perfusion (ILP) with melphalan (L-PAM), with or without tumor necrosis factor-alpha (TNF-alpha) to patients with multiple brain metastases, bone metastases, and spinal cord compression to achieve symptom relief and some shrinkage of the tumor with radiation therapy.

[00319]   In a specific embodiment, patients with pre-cancerous compound nevi are

15 administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant melanoma.

5.9.1.6. Treatment of Ovarian Cancer

[00320]   In specific embodiments, patients with ovarian cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment,

20 the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for ovarian cancer therapy including but not limited to: intraperitoneal radiation therapy, such as P^{32} therapy, total abdominal and pelvic radiation therapy, cisplatin, the combination of paclitaxel (Taxol) or docetaxel (Taxotere) and cisplatin or carboplatin, the combination of cyclophosphamide and cisplatin, the

25 combination of cyclophosphamide and carboplatin, the combination of 5-FU and leucovorin, etoposide, liposomal doxorubicin, gemcitabine or topotecan. It is contemplated that an effective amount of one or more EphA2 vaccines of the invention is administered in combination with the administration Taxol for patients with platinum-refractory disease. Included is the treatment of patients with refractory ovarian cancer including administration

30 of: ifosfamide in patients with disease that is platinum-refractory, hexamethylmelamine (HMM) as salvage chemotherapy after failure of cisplatin-based combination regimens, and tamoxifen in patients with detectable levels of cytoplasmic estrogen receptor on their tumors.

5.9.1.7. Treatment of Lung Cancers

[00321] In specific embodiments, patients with small lung cell cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for lung cancer therapy including

5   but not limited to: thoracic radiation therapy, cisplatin, vincristine, doxorubicin, and etoposide, alone or in combination, the combination of cyclophosphamide, doxorubicin, vincristine/etoposide, and cisplatin (CAV/EP), local palliation with endobronchial laser therapy, endobronchial stents, and/or brachytherapy.

[00322] In other specific embodiments, patients with non-small lung cell cancer are

10   administered an effective amount of one or more EphA2 vaccines of the invention in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: palliative radiation therapy, the combination of cisplatin, vinblastine and mitomycin, the combination of cisplatin and vinorelbine, paclitaxel, docetaxel or gemcitabine, the combination of carboplatin and paclitaxel,

15   interstitial radiation therapy for endobronchial lesions or stereotactic radiosurgery.

5.9.1.8. Treatment of T Cell Malignancies

[00323] In specific embodiments, patients with T cell malignancies, such as leukemias and lymphomas (see, e.g., section 5.9.1.1), are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the EphA2

20   vaccines of the invention can be administered in combination with an effective amount of one or more other agents useful for the prevention, treatment or amelioration of cancer, particularly T cell malignancies or one or more symptoms thereof, said combination therapies comprising administering to a subject in need thereof a prophylactically or therapeutically effective amount of one or more EphA2 vaccines of the invention and a

25   prophylactically or therapeutically effective amount of one or more cancer therapies, including chemotherapies, hormonal therapies, biological therapies, immunotherapies, or radiation therapies.

[00324] In another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in

30   combination with one or more cancer chemotherapeutic agents, such as but not limited to: doxorubicin, epirubicin, cyclophosphamide, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, vinblastine, dacarbazine, nitrosoureas such as carmustine and lomustine, vinca alkaloids, platinum compounds, cisplatin, mitomycin, vinorelbine, gemcitabine, carboplatin,

35   hexamethylmelamine and/or topotecan. Such methods can optionally further comprise the

administration of other cancer therapies, such as but not limited to radiation therapy, biological therapies, hormonal therapies and/or surgery.

[00325]   In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in

5   combination with one or more types of radiation therapy, such as external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to

10   chemotherapies, biological therapies/immunotherapies, hormonal therapies and/or surgery.

[00326]   In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with one or more biological therapies/immunotherapies or hormonal therapies, such as tamoxifen, leuprolide or other LHRH agonists, non-steroidal antiandrogens

15   (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), estrogens (DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate), aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, ketoconazole, prednisone, interferon-α, interleukin-2, tumor necrosis factor-α, and/or melphalan. Biological therapies also included are cytokines such as but not limited to TNF

20   ligand family members such as TRAIL anti-cancer agonists that induce apoptosis, TRAIL antibodies that bind to TRAIL receptors 1 and 2 otherwise known as DR4 and DR5 (Death Domain Containing Receptors 4 and 5), as well as DR4 and DR5. TRAIL and TRAIL antibodies, ligands and receptors are known in the art and described in U.S. Patent Nos. 6,342,363, 6,284,236, 6,072,047 and 5,763,223. Such methods can optionally further

25   comprise the administration of other cancer therapies, such as but not limited to radiation therapy, chemotherapies, and/or surgery.

[00327]   In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with standard and experimental therapies of T cell malignancies. Standard and

30   experimental therapies of T cell malignancies that can be used in the methods and compositions of the invention include, but are not limited to, antibody therapy (e.g., Campath®, anti-Tac, HuM291 (humanized murine IgG2 monoclonal antibody against CD3), antibody drug conjugates (e.g., Mylotarg), radiolabeled monoclonal antibodies (e.g., Bexxar, Zevalin, Lym-1)), cytokine therapy, aggressive combination chemotherapy with or

35   without cytotoxic agents, purine analogs, hematopoietic stem cell transplantation, and T cell

mediated therapy (e.g., CD8+ T cells with anti-leukemic activity against target antigens including but not limited to leukemia specific proteins (e.g., bcr/abl, PML/RARa, EMV/AML-1), leukemia-associated proteins (e.g., proteinase 3, WT-1, h-TERT, hdm-2)). (See Riddell et el., 2002, Cancer Control, 9(2): 114-122; Dearden et al. , 2002, Medical

5 Oncology, 19, Suppl. S27-32; Waldmann et al. 2000, Hematology (Am Soc Hematol Educ Program):394 408).

5.9.2 Treatment or Prevention of Disorders Associated with Aberrant Angiogenesis

[00328]   EphA2 is as a marker of angiogenic blood vessels and plays a critical role in
10 angiogenesis or neovascularization (see, e.g., Ogawa et al., 2000, Oncogene. 19(52):6043-52; Hess et al., 2001, Cancer Res. 61(8):3250-5). Angiogenesis is characterized by the invasion, migration and proliferation of smooth muscle and endothelial cells. The growth of new blood vessels, or angiogenesis, contributes to pathological conditions such as diabetic retinopathy (Adonis et al., 1994, Amer. J. Ophthal. 118:445), rheumatoid arthritis
15 (Peacock et al., 1992, J. Exp. Med., 175:1135) and osteoarthritis (Ondrick et al., 1992, Clin. Podiatr. Med. Surg. 9:185).

[00329]   The EphA2 vaccines of the invention may therefore be administered to a subject in need thereof to prevent, manage, treat or ameliorate a disorder associated with aberrant angiogenesis or one or more symptoms thereof.

20 [00330]   Disorders that are associated with or characterized by aberrant angiogenesis and may be prevented, treated, managed, or ameliorated with the EphA2 vaccines of the invention include, but are not limited to, neoplastic diseases (non-limiting examples are metastases of tumors and leukemia); diseases of ocular neovascularization (non-limiting examples are age-related macular degeneration, diabetic retinopathy, and retinopathy of
25 prematurity, vascular restenosis); skin diseases (non-limiting examples are infantile hemangiomas, verruca vulgaris, psoriasis, basal cell and squamous cell carcinomas, cutaneous melanoma, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa); arthritis (non-limiting examples are rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's
30 syndrome); gynecologic diseases (non-limiting examples are endometriosis, preeclampsia during pregnancy, carcinoma of the ovary, endometrium and cervix); and cardiovascular diseases (non-limiting examples are formation of atherosclerotic plaques, atherosclerosis and coronary artery disease).

[00331]   In specific embodiments, the disorders that are associated with or
35 characterized by aberrant angiogenesis and that may be prevented, treated, managed, or

ameliorated with the EphA2 vaccines of the invention include chronic articular rheumatism, psoriasis, diabetic retinopathy, neovascular glaucoma, macular degeneration, capillary proliferation in atherosclerotic plaques as well as cancers in which EphA2 is expressed in the vasculature. Such cancer disorders can include, for example, solid tumors, tumor metastasis, angiofibromas, retrolental, fibroplasia, hemangiomas, Kaposi's sarcoma.

[00332]   In certain embodiments, the EphA2 vaccines are employed in combination therapy regimens involving other therapies. Non-limiting examples of such therapies include analgesics, angiogenesis inhibitors, anti-cancer therapies and anti-inflammatory agents, in particular analgesics and angiogenesis inhibitors.

5.9.2.1. Patient Population

[00333]   The present invention encompasses methods for treating, managing, or preventing a disorder associated with aberrant angiogenesis or a symptom thereof, in a subject comprising administering one or more EphA2 vaccines. The methods of the invention comprise the administration of one or more EphA2 vaccines to patients suffering from or expected to suffer from (e.g., patients with a genetic predisposition for or patients that have previously suffered from) a disorder associated with aberrant angiogenesis. Such patients may have been previously treated or are currently being treated for the disorder. In accordance with the invention, an EphA2 vaccine may be used as any line of therapy, including, but not limited to, a first, second, third and fourth line of therapy. Further, in accordance with the invention, an EphA2 vaccine can be used before any adverse effects or intolerance of the EphA2 vaccine therapies occurs. The invention encompasses methods for administering one or more EphA2 vaccines of the invention to prevent the onset or recurrence of a disorder associated with aberrant angiogenesis.

[00334]   In one embodiment, the invention also provides methods of treatment or management of a disorder associated with aberrant angiogenesis as alternatives to current therapies. In a specific embodiment, the current therapy has proven or may prove too toxic (i.e., results in unacceptable or unbearable side effects) for the patient. In another embodiment, the patient has proven refractory to a current therapy. In such embodiments, the invention provides for the administration of one or more EphA2 vaccines of the invention without any other therapies for treating or managing the disorder associated with aberrant angiogenesis. In certain embodiments, one or more EphA2 vaccines of the invention can be administered to a patient in need thereof instead of another therapy to treat or manage a disorder associated with aberrant angiogenesis.

[00335]   The present invention also encompasses methods for administering one or more EphA2 vaccines of the invention to treat or ameliorate symptoms of a disorder

associated with aberrant angiogenesis in patients that are or have become refractory to non-EphA2 vaccine therapies. The determination of whether the symptoms are refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a therapy on affected cells in the disorder associated with aberrant

5   angiogenesis, or in patients that are or have become refractory to non-EphA2 vaccine therapies.

5.9.3   Other Therapies

[00336]   In some embodiments, therapy by administration of one or more EphA2 vaccines is combined with the administration of one or more therapies such as, but not

10   limited to, chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies. Prophylactic/therapeutic agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, including post-translationally modified proteins, peptides etc.; or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but

15   not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules. Prophylactic/therapeutic agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules.

[00337]   In a specific embodiment, the methods of the invention encompass

20   administration of an EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents, including antibodies, that are inhibitors of kinases such as, but not limited to, ABL, ACK, AFK, AKT (*e.g.*, AKT-1, AKT-2, and AKT-3), ALK, AMP-PK, ATM, Auroral, Aurora2, bARK1, bArk2, BLK, BMX, BTK, CAK, CaM kinase, CDC2, CDK, CK, COT, CTD, DNA-PK, EGF-R, ErbB-1, ErbB-2,

25   ErbB-3, ErbB-4, ERK (*e.g.*, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7), ERT-PK, FAK, FGR (*e.g.*, FGF1R, FGF2R), FLT (*e.g.*, FLT-1, FLT-2, FLT-3, FLT-4), FRK, FYN, GSK (*e.g.*, GSK1, GSK2, GSK3-alpha, GSK3-beta, GSK4, GSK5), G-protein coupled receptor kinases (GRKs), HCK, HER2, HKII, JAK (*e.g.*, JAK1, JAK2, JAK3, JAK4), JNK (*e.g.*, JNK1, JNK2, JNK3), KDR, KIT, IGF-1 receptor, IKK-1, IKK-2, INSR (insulin

30   receptor), IRAK1, IRAK2, IRK, ITK, LCK, LOK, LYN, MAPK, MAPKAPK-1, MAPKAPK-2, MEK, MET, MFPK, MHCK, MLCK, MLK3, NEU, NIK, PDGF receptor alpha, PDGF receptor beta, PHK, PI-3 kinase, PKA, PKB, PKC, PKG, PRK1, PYK2, p38 kinases, p135tyk2, p34cdc2, p42cdc2, p42mapk, p44mpk, RAF, RET, RIP, RIP-2, RK, RON, RS kinase, SRC, SYK, S6K, TAK1, TEC, TIE1, TIE2, TRKA, TXK, TYK2, UL13,

35   VEGFR1, VEGFR2, YES, YRK, ZAP-70, and all subtypes of these kinases (see *e.g.*,

Hardie and Hanks (1995) The Protein Kinase Facts Book, I and II, Academic Press, San

Diego, Calif.). In preferred embodiments, an EphA2 vaccine of the invention is

administered in combination with the administration of one or more

prophylactic/therapeutic agents that are inhibitors of Eph receptor kinases (*e.g.*, EphA2,

5   EphA4). In a most preferred embodiment, an EphA2 vaccine of the invention is

administered in combination with the administration of one or more

prophylactic/therapeutic agents that are inhibitors of EphA2.

[00338]   In a specific embodiment, the methods of the invention encompass

administration of an EphA2 vaccine of the invention in combination with the administration

10   of one or more therapeutic antibodies. Examples of therapeutic antibodies that can be used

in methods of the invention include but are not limited to AVASTIN® which is an anti-

VEGF antibody; antibodies that immunospecifically bind to EphA2 induce signal

transduction (*i.e.*, EphA2 agonistic antibodies); antibodies that immunospecifically bind to

EphrinA1; HERCEPTIN® (Trastuzumab) (Genentech, CA) which is a humanized anti-

15   HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer;

REOPRO® (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor on the

platelets for the prevention of clot formation; ZENAPAX® (daclizumab) (Roche

Pharmaceuticals, Switzerland) which is an immunosuppressive, humanized anti-CD25

monoclonal antibody for the prevention of acute renal allograft rejection; PANOREX™

20   which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo

Wellcome/Centocor); BEC2 which is a murine anti-idiotype (GD3 epitope) IgG antibody

(ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone

System); VITAXIN™ which is a humanized anti-$\alpha_v\beta_3$ integrin antibody (Applied Molecular

Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1

25   antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein

Design Lab/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1 antibody (IDEC

Pharm/Genentech, Roche/Zettyaku); LYMPHOCIDE™ which is a humanized anti-CD22

IgG antibody (Immunomedics); LYMPHOCIDE™ Y-90 (Immunomedics); Lymphoscan

(Tc-99m-labeled; radioimaging; Immunomedics); Nuvion (against CD3; Protein Design

30   Labs); CM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatied

anti-CD80 antibody (IDEC Pharm/Mitsubishi); ZEVALIN™ is a radiolabelled murine anti-

CD20 antibody (IDEC/Schering AG); IDEC-131 is a humanized anti-CD40L antibody

(IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a

primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-

35   CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5)

antibody (Alexion Pharm); D2E7 is a humanized anti-TNF-alpha antibody (CAT/BASF);
CDP870 is a humanized anti-TNF-alpha Fab fragment (Celltech); IDEC-151 is a primatized
anti-CD4 IgG1 antibody (IDEC Pharm/SmithKline Beecham); MDX-CD4 is a human anti-
CD4 IgG antibody (Medarex/Eisai/Genmab); CD20-sreptdavidin (+biotin-yttrium 90;

5   NeoRx); CDP571 is a humanized anti-TNF-alpha IgG4 antibody (Celltech); LDP-02 is a
humanized anti- $\alpha_v\beta_7$antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized
anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ is a humanized anti-CD40L IgG
antibody (Biogen); ANTEGREN™ is a humanized anti-VLA-4 IgG antibody (Elan); and
CAT-152 is a human anti-TGF-beta$_2$ antibody (Cambridge Ab Tech).

10   [00339]   In another specific embodiment, the methods of the invention encompass
administration of an EphA2 vaccine of the invention in combination with the administration
of one or more prophylactic/therapeutic agents that are angiogenesis inhibitors such as, but
not limited to: Angiostatin (plasminogen fragment); antiangiogenic antithrombin III;
Angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab (AVASTIN™); BMS-275291;

15   cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3;
Combretastatin A-4; Endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta;
Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; Human chorionic
gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein
(IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase

20   inhibitors (TIMPs); 2-Methoxyestradiol; MMl 270 (CGS 27023A); MoAb IMC-1C11;
Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; Plasminogen activator
inhibitor; Platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related
protein (PRP); PTK 787/ZK 222594; Retinoids; Solimastat; Squalamine; SS 3304; SU
5416; SU6668; SU11248; Tetrahydrocortisol-S; tetrathiomolybdate; thalidomide;

25   Thrombospondin-1 (TSP-1); TNP-470; Transforming growth factor-beta (TGF-β);
Vasculostatin; Vasostatin (calreticulin fragment); ZD6126; ZD6474; farnesyl transferase
inhibitors (FTI); and bisphosphonates.

[00340]   In another specific embodiment, the methods of the invention encompass
administration of an EphA2 vaccine of the invention in combination with the administration

30   of one or more prophylactic/therapeutic agents that are anti-cancer agents such as, but not
limited to: acivicin, aclarubicin, acodazole hydrochloride, acronine, adozelesin,
aldesleukin, altretamine, ambomycin, ametantrone acetate, aminoglutethimide, amsacrine,
anastrozole, anthramycin, asparaginase, asperlin, azacitidine, azetepa, azotomycin,
batimastat, benzodepa, bicalutamide, bisantrene hydrochloride, bisnafide dimesylate,

35   bizelesin, bleomycin sulfate, brequinar sodium, bropirimine, busulfan, cactinomycin,

calusterone, caracemide, carbetimer, carboplatin, carmustine, carubicin hydrochloride, carzelesin, cedefingol, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin hydrochloride, decarbazine, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone,

5   docetaxel, doxorubicin, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, duazomycin, edatrexate, eflornithine hydrochloride, elsamitrucin, enloplatin, enpromate, soluble EphrinA1, EphrinA1-Fc polypeptides, EphA2-Fc polypeptides, EphA2 antisense, EphrinA1 antisense, epipropidine, epirubicin hydrochloride, erbulozole, esorubicin hydrochloride, estramustine, estramustine phosphate

10   sodium, etanidazole, etoposide, etoposide phosphate, etoprine, fadrozole hydrochloride, fazarabine, fenretinide, floxuridine, fludarabine phosphate, fluorouracil, flurocitabine, fosquidone, fostriecin sodium, gemcitabine, gemcitabine hydrochloride, hydroxyurea, idarubicin hydrochloride, ifosfamide, ilmofosine, interleukin 2 (including recombinant interleukin 2, or rIL2), interferon alpha-2a, interferon alpha-2b, interferon alpha-n1,

15   interferon alpha-n3, interferon beta-I a, interferon gamma-I b, iproplatin, irinotecan hydrochloride, lanreotide acetate, letrozole, leuprolide acetate, liarozole hydrochloride, lometrexol sodium, lomustine, losoxantrone hydrochloride, masoprocol, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, methotrexate sodium, metoprine, meturedepa,

20   mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone hydrochloride, mycophenolic acid, nitrosoureas, nocodazole, nogalamycin, ormaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin sulfate, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine

25   hydrochloride, puromycin, puromycin hydrochloride, pyrazofurin, riboprine, rogletimide, safingol, safingol hydrochloride, semustine, simtrazene, sparfosate sodium, sparsomycin, spirogermanium hydrochloride, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tecogalan sodium, tegafur, teloxantrone hydrochloride, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin,

30   tirapazamine, toremifene citrate, trastuzumab (HERCEPTIN™), trestolone acetate, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tubulozole hydrochloride, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate, vinleurosine sulfate, vinorelbine tartrate, vinrosidine sulfate, vinzolidine sulfate, vorozole,

35   zeniplatin, zinostatin, zorubicin hydrochloride. Other anti-cancer drugs include, but are not

limited to: 20-epi-1,25 dihydroxyvitamin D3, 5-ethynyluracil, abiraterone, aclarubicin,
acylfulvene, adecypenol, adozelesin, aldesleukin, ALL-TK antagonists, altretamine,
ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide,
anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix,

5   anti-dorsalizing morphogenetic protein-1, antiandrogens, antiestrogens, antineoplaston,
aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-
CDP-DL-PTBA, arginine deaminase, asulacrine, atamestane, atrimustine, axinastatin 1,
axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives,
balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta

10   lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, bFGF inhibitor,
bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate,
bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin
derivatives, canarypox IL-2, capecitabine, carboxamide-amino-triazole,
carboxyamidotriazole, CaRest M3, CARN 700, cartilage derived inhibitor, carzelesin,

15   casein kinase inhibitors (ICOS), castanospermine, cecropin B, cetrorelix, chloroquinoxaline
sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene analogues, clotrimazole,
collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin,
crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A,
cyclopentanthraquinones, cycloplatam, cypemycin, cytarabine ocfosfate, cytolytic factor,

20   cytostatin, dacliximab, decitabine, dehydrodidemnin B, deslorelin, dexamethasone,
dexifosfamide, dexrazoxane, dexverapamil, diaziquone, didemnin B, didox,
diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl
spiromustine, docetaxel, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol,
duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, eflornithine, elemene,

25   emitefur, epirubicin, epristeride, estramustine analogue, estrogen agonists, estrogen
antagonists, etanidazole, etoposide phosphate, exemestane, fadrozole, fazarabine,
fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine,
fluorodaunorunicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine,
gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors,

30   gemcitabine, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide,
hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ilmofosine, ilomastat,
imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1
receptor inhibitor, interferon agonists, interferons, interleukins, iobenguane,
iododoxorubicin, ipomeanol, iroplact, irsogladine, isobengazole, isohomohalicondrin B,

35   itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin,

lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte
alpha interferon, leuprolide+estrogen+progesterone, leuprorelin, levamisole, liarozole,
linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds,
lissoclinamide 7, lobaplatin, lombricine, lometrexol, lonidamine, losoxantrone, lovastatin,

5   loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine,
mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix
metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase,
metoclopramide, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double
stranded RNA, mitoguazone, mitolactol, mitomycin analogues, mitonafide, mitotoxin

10   fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, human
chorionic gonadotrophin, monophosphoryl lipid A+myobacterium cell wall sk, mopidamol,
multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard
anticancer agent, mycaperoxide B, mycobacterial cell wall extract, myriaporone, N-
acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, naloxone+pentazocine,

15   napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral
endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant,
nitrullyn, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone,
ondansetron, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin,
oxaunomycin, paclitaxel, paclitaxel analogues, paclitaxel derivatives, palauamine,

20   palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine,
pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentrozole, perflubron,
perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors,
picibanil, pilocarpine hydrochloride, pirarubicin, piritrexim, placetin A, placetin B,
plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine

25   complex, porfimer sodium, porfiromycin, prednisone, propyl bis-acridone, prostaglandin J2,
proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor,
protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine
nucleoside phosphorylase inhibitors, purpurins, pyrazoloacridine, pyridoxylated
hemoglobin polyoxyethylene conjugate, raf antagonists, raltitrexed, ramosetron, ras farnesyl

30   protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated,
rhenium Re 186 etidronate, rhizoxin, ribozymes, RII retinamide, rogletimide, rohitukine,
romurtide, roquinimex, rubiginone B1, ruboxyl, safingol, saintopin, SarCNU, sarcophytol
A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense
oligonucleotides, signal transduction inhibitors, signal transduction modulators, single chain

35   antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate,

solverol, somatomedin binding protein, sonermin, sparfosic acid, spicamycin D,

spiromustine, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell

division inhibitors, stipiamide, stromelysin inhibitors, sulfinosine, superactive vasoactive

intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic

5   glycosaminoglycans, tallimustine, tamoxifen methiodide, tauromustine, taxol, tazarotene,

tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, temoporfin,

temozolomide, teniposide, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide,

thiocoraline, thioguanine, thrombopoietin, thrombopoietin mimetic, thymalfasin,

thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tin ethyl

10   etiopurpurin, tirapazamine, titanocene bichloride, topsentin, toremifene, totipotent stem cell

factor, translation inhibitors, tretinoin, triacetyluridine, triciribine, trimetrexate, triptorelin,

tropisetron, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex,

urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists,

vapreotide, variolin B, vector system, erythrocyte gene therapy, velaresol, VEGF

15   antagonists (e.g., anti-VEGF antibodies), VEGFR antagonists (e.g., anti-VEGFR

antibodies), veramine, verdins, verteporfin, vinorelbine, vinxaltine, vitaxin, vorozole,

zanoterone, zeniplatin, zilascorb, and zinostatin stimalamer. Preferred additional anti-

cancer drugs are 5-fluorouracil and leucovorin.

[00341]   In more particular embodiments, the present invention also comprises the

20   administration of one or more EphA2 vaccines of the invention in combination with the

administration of one or more therapies such as, but not limited to anti-cancer agents such

as those disclosed in Table 5 below, preferably for the treatment of breast, ovary,

melanoma, prostate, colon and lung cancers as described above.

TABLE 5

Therapeutic Agent	Administration	Dose	Intervals
doxorubicin hydrochloride (Adriamycin RDF® and Adriamycin PFS®)	Intravenous	60-75 mg/m^2 on Day 1	21 day intervals
epirubicin hydrochloride (Ellence™)	Intravenous	100-120 mg/m^2 on Day 1 of each cycle or divided equally and given on Days 1-8 of the cycle	3-4 week cycles
fluorousacil	Intravenous	How supplied: 5 ml and 10 ml vials (containing 250 and 500 mg flourouracil respectively)	
docetaxel (Taxotere®)	Intravenous	60- 100 mg/m^2 over 1 hour	Once every 3 weeks

Therapeutic Agent	Administration	Dose	Intervals
paclitaxel (Taxol®)	Intravenous	175 mg/m² over 3 hours	Every 3 weeks for 4 courses (administered sequentially to doxorubicin-containing combination chemotherapy)
tamoxifen citrate (Nolvadex®)	Oral (tablet)	20-40 mg Dosages greater than 20 mg should be given in divided doses (morning and evening)	Daily
leucovorin calcium for injection	Intravenous or intramuscular injection	How supplied: 350 mg vial	Dosage is unclear from text. PDR 3610
luprolide acetate (Lupron®)	Single subcutaneous injection	1 mg (0.2 ml or 20 unit mark)	Once a day
flutamide (Eulexin®)	Oral (capsule)	250 mg (capsules contain 125 mg flutamide each)	3 times a day at 8 hour intervals (total daily dosage 750 mg)
nilutamide (Nilandron®)	Oral (tablet)	300 mg or 150 mg (tablets contain 50 or 150 mg nilutamide each)	300 mg once a day for 30 days followed by 150 mg once a day
bicalutamide (Casodex®)	Oral (tablet)	50 mg (tablets contain 50 mg bicalutamide each)	Once a day
progesterone	Injection	USP in sesame oil 50 mg/ml	
ketoconazole (Nizoral®)	Cream	2% cream applied once or twice daily depending on symptoms	
prednisone	Oral (tablet)	Initial dosage may vary from 5 mg to 60 mg per day depending on the specific disease entity being treated.	
estramustine phosphate sodium (Emcyt®)	Oral (capsule)	14 mg/ kg of body weight (*i.e.* one 140 mg capsule for each 10 kg or 22 lb of body weight)	Daily given in 3 or 4 divided doses
etoposide or VP-16	Intravenous	5 ml of 20 mg/ ml solution (100 mg)	
dacarbazine (DTIC-Dome®)	Intravenous	2-4.5 mg/knowing	Once a day for 10 days. May be repeated at 4 week intervals
polifeprosan 20 with carmustine implant (BCNU) (nitrosourea) (Gliadel®)	wafer placed in resection cavity	8 wafers, each containing 7.7 mg of carmustine, for a total of 61.6 mg, if size and shape of resection cavity allows	
cisplatin	Injection	How supplied: solution of 1 mg/ml in multi-dose vials of 50mL and 100mL	
mitomycin	Injection	supplied in 5 mg and 20 mg vials (containing 5 mg and 20 mg mitomycin)	

Therapeutic Agent	Administration	Dose	Intervals
gemcitabine HCl (Gemzar®)	Intravenous	For NSCLC- 2 schedules have been investigated and the optimum schedule has not been determined 4 week schedule- administration intravenously at 1000 mg/m² over 30 minutes on 3 week schedule- Gemzar administered intravenously at 1250 mg/m² over 30 minutes	4 week schedule- Days 1,8 and 15 of each 28-day cycle. Cisplatin intravenously at 100 mg/m² on day 1 after the infusion of Gemzar. 3 week schedule- Days 1 and 8 of each 21 day cycle. Cisplatin at dosage of 100 mg/m² administered intravenously after administration of Gemzar on day 1.
carboplatin (Paraplatin®)	Intravenous	Single agent therapy: 360 mg/m² I.V. on day 1 (infusion lasting 15 minutes or longer) Other dosage calculations: Combination therapy with cyclophosphamide, Dose adjustment recommendations, Formula dosing, etc.	Every 4 weeks
Ifosamide (Ifex®)	Intravenous	1.2 g/m² daily	5 consecutive days Repeat every 3 weeks or after recovery from hematologic toxicity
Topotecan hydrochloride (Hycamtin®)	Intravenous	1.5 mg/m² by intravenous infusion over 30 minutes daily	5 consecutive days, starting on day 1 of 21 day course

[00342]   The invention also encompasses administration of the EphA2 vaccines of the invention in combination with radiation therapy comprising the use of x-rays, gamma rays and other sources of radiation to destroy the cancer cells. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the

5   radiation is directed from a remote source. In other preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass.

[00343]   In a specific embodiment, the methods of the invention encompass administration of an EphA2 vaccine of the invention in combination with the administration

10   of one or more anti-inflammatory agents. Any anti-inflammatory agent, including agents useful in therapies for inflammatory disorders, well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, anticholinergics (e.g., atropine sulfate, atropine methylnitrate, and

15   ipratropium bromide (ATROVENT™)), beta2-agonists (e.g., abuterol (VENTOLIN™ and PROVENTIL™), bitolterol (TORNALATE™), levalbuterol (XOPONEX™),

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metaproterenol (ALUPENT™), pirbuterol (MAXAIR™), terbutlaine (BRETHAIRE™ and BRETHINE™), albuterol (PROVENTIL™, REPETABS™, and VOLMAX™), formoterol (FORADIL AEROLIZER™), and salmeterol (SEREVENT™ and SEREVENT DISKUS™)), and methylxanthines (*e.g.*, theophylline (UNIPHYL™, THEO-DUR™, SLO-

5   BID™, AND TEHO-42™)). Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX™), diclofenac (VOLTAREN™), etodolac (LODINE™), fenoprofen (NALFON™), indomethacin (INDOCIN™), ketoralac (TORADOL™), oxaprozin (DAYPRO™), nabumentone (RELAFEN™), sulindac (CLINORIL™), tolmentin (TOLECTIN™), rofecoxib (VIOXX™), naproxen (ALEVE™,

10   NAPROSYN™), ketoprofen (ACTRON™) and nabumetone (RELAFEN™). Such NSAIDs function by inhibiting a cyclooxgenase enzyme (*e.g.*, COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRON™), corticosteroids (*e.g.*, methylprednisolone (MEDROL™)), cortisone, hydrocortisone, prednisone

15   (PREDNISONE™ and DELTASONE™), prednisolone (PRELONE™ and PEDIAPRED™), triamcinolone, azulfidine, and inhibitors of eicosanoids (*e.g.*, prostaglandins, thromboxanes, and leukotrienes (*see* Table 6, *infra*, for non-limiting examples of leukotriene and typical dosages of such agents)).

[00344]   In certain embodiments, the anti-inflammatory agent is an agent useful in the

20   prevention, management, treatment, and/or amelioration of asthma or one or more symptoms thereof. Non-limiting examples of such agents include adrenergic stimulants (*e.g.*, catecholamines (*e.g.*, epinephrine, isoproterenol, and isoetharine), resorcinols (*e.g.*, metaproterenol, terbutaline, and fenoterol), and saligenins (*e.g.*, salbutamol)), adrenocorticoids, blucocorticoids, corticosteroids (*e.g.*, beclomethadonse, budesonide,

25   flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, and prednisone), other steroids, beta2-agonists (*e.g.*, albtuerol, bitolterol, fenoterol, isoetharine, metaproterenol, pirbuterol, salbutamol, terbutaline, formoterol, salmeterol, and albutamol terbutaline), anti-cholinergics (*e.g.*, ipratropium bromide and oxitropium bromide), IL-4 antagonists (including antibodies), IL-5 antagonists (including antibodies), IL-13

30   antagonists (including antibodies), PDE4-inhibitor, NF-Kappa-β inhibitor, VLA-4 inhibitor, CpG, anti-CD23, selectin antagonists (TBC 1269), mast cell protease inhibitors (*e.g.*, tryptase kinase inhibitors (*e.g.*, GW-45, GW-58, and genisteine), phosphatidylinositide-3' (PI3)-kinase inhibitors (*e.g.*, calphostin C), and other kinase inhibitors (*e.g.*, staurosporine) (*see* Temkin *et al.*, 2002 J Immunol 169(5):2662-2669; Vosseller *et al.*, 1997 Mol. Biol.

35   Cell 8(5):909-922; and Nagai *et al.*, 1995 Biochem Biophys Res Commun 208(2):576-

581)), a C3 receptor antagonists (including antibodies), immunosuppressant agents (*e.g.*, methotrexate and gold salts), mast cell modulators (*e.g.*, cromolyn sodium (INTAL™) and nedocromil sodium (TILADE™)), and mucolytic agents (*e.g.*, acetylcysteine)). In a specific embodiment, the anti-inflammatory agent is a leukotriene inhibitor (*e.g.*,

5   montelukast (SINGULAIR™), zafirlukast (ACCOLATE™), pranlukast (ONON™), or zileuton (ZYFLO™) (*see* Table 6)).

Table 6. Leukotriene Inhibitors for Asthma Therapy

Leukotriene Modifier	Usual Daily Dosage
Montelukast (SINGULAIR™)	4 mg for 2-5 years old 5 mg for 6 to 15 years old 10mg for 15 years and older
Zafirlukast (ACCOLATE™)	10 mg b.i.d. for 5 to 12 years old twice daily 20 mg b.i.d. for 12 years or older twice daily
Pranlukast (ONON™)	Only avialable in Asia
Zyleuton (ZYFLO™)	600 mg four times a day for 12 years and older

10   Table 7. H₁ Antihistamines

Chemical class and representative drugs	Usual daily dosage

Ethanolamine
   Diphehydramine
   Clemastine
	
	25-50 mg every 4-6 hours
	0.34-2.68 mg every 12 hours

Ethylenediamine Tripelennamine	 25-50 mg every 4-6 hours

Alkylamine
   Brompheniramine
 	
   Chlorpheniramine
 	
   Triprolidine (1.25 mg/5ml)
	
	4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour
	4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour
	2.5 mg every 4-6 hours

Phenothiazine Promethazine	 25 mg at bedtime
Piperazine Hydroxyzine	 25 mg every 6-8 hours

Piperidines
   Astemizole (nonsedating)
   Azatadine
   Cetirzine
   Cyproheptadine
   Fexofenadine (nonsedating)
   Loratidine (nonsedating)
	
	10 mg/day
	1-2 mg every 12 hours
	10 mg/day
	4 mg every 6-8 hour
	60 mg every 12 hours
	10 mg every 24 hours

[00345]   Cancer therapies, as well as therapies for hyperproliferative cell disorders other than cancer and their dosages, routes of administration and recommended usage are

known in the art and have been described in such literature as the *Physician's Desk* ·
Reference (56th ed., 2002, 57th ed., 2003, and 58th ed., 2004).

5.9.4   Passive Immunization

[00346]   The EphA2 antigenic peptides of the invention may be used to generate
antibodies in a suitable host for use in passive immunization methods, diagnostic
immunoassays and the generation of anti-idiotypic antibodies. Any suitable host may be
used to generate an anti-EphA2 antibody including, but not limited to, mice, rabbits,
chimpanzees, and humans. The antibodies may be isolated by standard techniques (e.g.,
immunoaffinity chromatography, centrifugation, precipitation, etc.).

[00347]   The antibodies generated in a suitable host may be further modified through
methods known to one of ordinary skill in the art, including, but not limited to, introducing
mutations. In certain embodiments, the antibodies are modified, *e.g.*, mutations are
introduced, to increase affinity and/or half-life or humanize the antibody. Standard
techniques known to those of skill in the art can be used to introduce mutations (*e.g.*,
deletions, additions, and/or substitutions) in the nucleotide sequence encoding an antibody
of the invention, including, for example, site-directed mutagenesis and PCR-mediated
mutagenesis which results in amino acid substitutions. Preferably, the derivatives include
less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15
amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid
substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or
less than 2 amino acid substitutions relative to the original molecule. In a preferred
embodiment, the derivatives have conservative amino acid substitutions are made at one or
more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not
critical for the antibody to immunospecifically bind to an EphA2 antigen). A "conservative
amino acid substitution" is one in which the amino acid residue is replaced with an amino
acid residue having a side chain with a similar charge. Families of amino acid residues
having side chains with similar charges have been defined in the art. These families include
amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*,
aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine,
glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine,
leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side
chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine,
phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly
along all or part of the coding sequence, such as by saturation mutagenesis, and the
resultant mutants can be screened for biological activity to identify mutants that retain

activity. Following mutagenesis, the encoded antibody can be expressed and the activity of the antibody can be determined.

[00348] The generated antibodies can also be used in the diagnostic immunoassays. The antibodies may also be used to diagnose disease, monitor treatment and/or disease progression. For example, the antibodies may be used to diagnose and/or prognose the malignancy of cancer. See, *e.g.*, U.S. Patent Application Serial No. 10/436,782, incorporated by reference herein in its entirety.

[00352] Any immunoassay system know in the art, such as those listed *supra*, may be used for this purpose including but not limited to competitive and noncompetitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme-linked immunosorbent assays), "sandwich" immunoassays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few.

[00353] The EphA2 vaccines of the present invention can be used to produce antibodies for use in passive immunization. The antibodies used in passive immunization of a subject use preferably isolated. Alternatively, sera from an immunized host is used. In a preferred embodiment, an antibody administered to a human subject is human or humanized.

[00354] Antibodies that immunospecifically bind to EphA2 may be used to treat a hyperproliferative disorder, a disorder associated with the overexpression of EphA2 and/or a disorder associated with or involving aberrant angiogenesis. Thus, the invention provides methods of treating, preventing, and/or managing a hyperproliferative disorder (*e.g.*, cancer), a disorder associated with EphA2 overexpression and/or a disorder associated with or involving aberrant angiogenesis, the methods comprising administering to a subject (preferably, a human) an effective amount of an antibody that immunospecifically binds to EphA2 wherein the antibody was generated by immunizing a host with an EphA2 vaccine.

[00355] In accordance with the invention, such methods may further comprise the administration of an effective amount of a therapy other than the antibody. For example, the antibody may be administered in combination with an EphA2 vaccine and/or another type of therapy (See, *e.g.* Section 5.9.3, *infra*, regarding non-limiting examples of other types of therapies).

[00356] The antibodies generated by the vaccines of the present invention can also be used in the production of antiidiotypic antibody. The antiidiotypic antibody can then in turn be used for immunization, in order to produce a subpopulation of antibodies that bind the

initial antigen of the pathogenic microorganism (Jerne, 1974, Ann. Immunol. (Paris) 125c: 373; Jerne et al., 1982, EMBO J. 234).

[00357]   In immunization procedures, the amount of immunogen to be used and the immunization schedule will be determined by a physician skilled in the art and will be administered by reference to the immune response and antibody titers of the subject.

[00358]   In one embodiment, a composition of the invention comprises an antibody obtained by active immunization of a suitable host. In another embodiment, a composition of the invention comprise a modified antibody obtained by active immunization of a suitable host. In a preferred embodiment, the modified antibody is humanized.

5.10 Biological Activity

[00359]   Toxicity and efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Prophylactic and/or therapeutic agents that exhibit large therapeutic indices are preferred. While prophylactic and/or therapeutic agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00360]   The data obtained from the animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the vaccine or test compound that achieves a half-maximal inhibition of symptoms) as determined in animal studies. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00361]   The anti-cancer activity of the therapies used in accordance with the present invention also can be determined by using various experimental animal models for the study of cancer, such as an immunocompetent mouse model, e.g., Balb/c or C57/Bl/6, or

transgenic mice where a mouse EphA2 is replaced with the human EphA2, mouse models

to which murine tumor cell lines engineered to express human EphA2 are administered,

animal models described in Section 6 *infra*, or any animal model (including hamsters,

rabbits, etc.) known in the art and described in *Relevance of Tumor Models for Anticancer*

Drug Development (1999, eds. Fiebig and Burger); *Contributions to Oncology* (1999,

Karger); *The Nude Mouse in Oncology Research* (1991, eds. Boven and Winograd); and

Anticancer Drug Development Guide (1997 ed. Teicher), herein incorporated by reference

in their entireties.

[00362]   In certain embodiments, the therapies of the present invention result in

decreased tumor volume (*e.g.*, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80,

85, 90, 95%, or 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold less)

relative to a control in an animal, preferably, a mouse, model for cancer not treated with the

therapies of the invention.

[00363]   Compounds for use in therapy can also be tested in other suitable animal

model systems prior to testing in humans, including but not limited to in rats, mice, chicken,

cows, monkeys, rabbits, hamsters, etc., for example, the animal models described above.

The compounds can then be used in the appropriate clinical trials.

[00364]   The EphA2 vaccines, compositions comprising an EphA2 antigenic

fragment, or combination therapies of the invention are preferably tested *in vitro* and then *in*

vivo for the desired therapeutic or prophylactic activity prior to use in humans. For

example, assays which can be used to determine whether administration of a specific

prophylactic or therapeutic composition is indicated include cell culture assays in which a

patient tissue sample is grown in culture and exposed to, or otherwise contacted with, a

prophylactic or therapeutic composition, and the effect of such composition upon the tissue

sample is observed. The tissue sample can be obtained by biopsy from the patient. This

test allows the identification of the therapeutically most effective therapy (*e.g.*, prophylactic

or therapeutic agent) for each individual patient. In various specific embodiments, *in vitro*

assays can be carried out with representative cells of cell types involved a disease or

disorder associated with or characterized by increased expression of EphA2 to determine if

a prophylactic or therapeutic composition of the invention has a desired effect upon such

cell types.

[00365]   For example, the effect of an EphA2 vaccine, a composition comprising an

EphA2 antigenic fragment, or a combination therapy of the invention on peripheral blood

lymphocyte counts can be monitored/assessed using standard techniques known to one of

skill in the art. Peripheral blood lymphocytes counts in a subject can be determined by,

e.g., obtaining a sample of peripheral blood from said subject, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, Ficoll-Hypaque (Pharmacia) gradient centrifugation, and counting the lymphocytes using trypan blue. Peripheral blood T-cell counts in subject can be determined by, *e.g.*, separating the

5   lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, a use of Ficoll-Hypaque (Pharmacia) gradient centrifugation, labeling the T-cells with an antibody directed to a T-cell antigen which is conjugated to FITC or phycoerythrin, and measuring the number of T-cells by FACS.

[00366]   Further, any assays known to those skilled in the art can be used to evaluate

10   the prophylactic and/or therapeutic utility of the vaccines and combinatorial therapies disclosed herein for treatment or prevention of hyperproliferative disorders such as cancer.

5.11 Vaccine Compositions

[00367]   The compositions of the invention include bulk drug compositions useful in the manufacture of non-pharmaceutical compositions (*e.g.*, impure or non-sterile

15   compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention

20   comprise a prophylactically or therapeutically effective amount of one or more EphA2 vaccines of the invention. The EphA2 vaccines of the invention may comprise one or more EphA2 antigenic peptides of the invention and a pharmaceutically acceptable carrier, one or more EphA2 antigenic peptide expression vehicles of the invention and a pharmaceutically acceptable carrier, or one or more antigen presenting cells sensitized with an EphA2

25   antigenic peptide and a pharmaceutically acceptable carrier.

[00368]   Where an EphA2 vaccine of the invention comprises an EphA2 antigenic peptides, the EphA2 antigenic peptide of the invention can be modified. For example, in certain embodiments, the EphA2 antigenic peptide may be formulated with lipid as a lipopeptide or linked to a carrier molecule (and/or polymerized).

30   [00369]   In a specific embodiment, a composition of the invention comprises an EphA2 vaccine and an additional prophylactic or therapeutic, *e.g.*, anti-cancer, agent. In accordance with this embodiment, the composition may further comprise a pharmaceutically acceptable carrier.

[00370]   In a specific embodiment, the term "pharmaceutically acceptable" means

35   approved by a regulatory agency of the Federal or a state government or listed in the U.S.

Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete) or, more preferably, MF59C.1 adjuvant available from Chiron, Emeryville, CA), excipient, or vehicle with which the therapeutic is administered.

5   Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable

10   solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions,

15   suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00371]   Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or

20   sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

25   [00372]   The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

30   [00373]   Various delivery systems are known and can be used to administer an EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and a prophylactic agent or therapeutic agent useful for preventing or treating cancer, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the EphA2 antigenic peptide, receptor-mediated endocytosis (see, e.g., Wu and

35   Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a

retroviral or other vector, etc. Methods of administering an EphA2 vaccine or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal,

5   inhaled, and oral routes). In a specific embodiment, an EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent are administered intramuscularly, intravenously, or subcutaneously. The EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent may be administered by any convenient route, for example by infusion or

10   bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00374]   In a specific embodiment, it may be desirable to administer the EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and

15   prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the

20   site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

[00375]   In yet another embodiment, the EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton,

25   1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the EphA2 antigenic peptides of the invention (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product

30   Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 7 1:105); U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; 5,128,326; International Publication Nos. WO 99/15154 and WO 99/20253. Examples of

35   polymers used in sustained release formulations include, but are not limited to, poly(2-

hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred

5   embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

10   **[00376]**   Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning et al., 1996, *Radiotherapy & Oncology* 39:179-189;

15   Song et al., 1995, *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek et al., 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam et al., 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760, each of which is incorporated herein by reference in its entirety.

5.11.1 Formulations

20   **[00377]**   Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

[00378]   Thus, the EphA2 antigenic peptides of the invention and their physiologically acceptable salts and solvates (or EphA2 antigenic peptide expression

25   vehicles or antigen presenting cells sensitized with an EphA2 antigenic peptide) may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, parenteral or mucosal (such as buccal, vaginal, rectal, sublingual) administration. In a preferred embodiment, local or systemic parenteral administration is used.

30   **[00379]**   For oral administration, the vaccine may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*,

35   potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate).

The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with

5   pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening

10 agents as appropriate.

[00380]   Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[00381]   For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

15 [00382]   For administration by inhalation, the prophylactic or therapeutic agents for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized

20 aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00383]   The EphA2 vaccine may be formulated for parenteral administration by

25 injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder

30 form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00384]   The vaccines of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00385]   In addition to the formulations described previously, the prophylactic or

35 therapeutic agents may also be formulated as a depot preparation. Such long acting

formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the prophylactic or therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble

5   derivatives, for example, as a sparingly soluble salt.

[00386]   The invention also provides that an EphA2 vaccine of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity. In one embodiment, the vaccine is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*,

10   with water or saline to the appropriate concentration for administration to a subject.

[00387]   In a preferred embodiment of the invention, the formulation and administration of various chemotherapeutic, biological/immunotherapeutic and hormonal therapeutic agents for use in combination with the vaccine of the invention are known in the art and often described in the *Physician's Desk Reference, 56th ed.* (2002). For instance, in

15   certain specific embodiments of the invention, the agents can be formulated and supplied as known to one or ordinary skill in the art.

[00388]   In other embodiments of the invention, radiation therapy agents such as radioactive isotopes can be given orally as liquids in capsules or as a drink. Radioactive isotopes can also be formulated for intravenous injections. The skilled oncologist can

20   determine the preferred formulation and route of administration.

[00389]   In certain embodiments the EphA2 antigenic peptides and anti-idiotypic antibodies of the invention are formulated at 1 mg/ml, 5 mg/ml, 10 mg/ml, and 25 mg/ml for intravenous injections and at 5 mg/ml, 10 mg/ml, and 80 mg/ml for repeated subcutaneous administration and intramuscular injection.

25   [00390]   Where the EphA2 vaccine is a bacterial vaccine, the vaccine can be formulated at amounts ranging between approximately 1×10^2 CFU/ml to approximately 1×10^{12} CFU/ml, for example at 1×10^2 CFU/ml, 5×10^2 CFU/ml, 1×10^3 CFU/ml, 5×10^3 CFU/ml, 1×10^4 CFU/ml, 5×10^4 CFU/ml, 1×10^5 CFU/ml, 5×10^5 CFU/ml, 1×10^6 CFU/ml, 5×10^6 CFU/ml, 1×10^7 CFU/ml, 5×10^7 CFU/ml, 1×10^8 CFU/ml, 5×10^8 CFU/ml, 1×10^9

30   CFU/ml, 5×10^9 CFU/ml, 1×10^{10} CFU/ml, 5×10^{10} CFU/ml, 1×10^{11} CFU/ml, 5×10^{11} CFU/ml, or 1×10^{12} CFU/ml.

[00391]   The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or

35   dispenser device may be accompanied by instructions for administration.

5.11.2 Dosages

[00392]   The amount of the composition of the invention which will be effective in

the treatment, prevention or management of cancer can be determined by standard research

techniques. For example, the dosage of the EphA2 vaccine of the invention which will be

5   effective in the treatment, prevention or management of cancer can be determined by

administering the composition to an animal model such as, *e.g.*, the animal models

disclosed herein or known to those skilled in the art. In addition, *in vitro* assays may

optionally be employed to help identify optimal dosage ranges.

[00393]   Selection of the preferred effective dose can be determined (*e.g.*, via clinical

10   trials) by a skilled artisan based upon the consideration of several factors which will be

known to one of ordinary skill in the art. Such factors include the disease to be treated or

prevented, the symptoms involved, the patient's body mass, the patient's immune status and

other factors known by the skilled artisan to reflect the accuracy of administered

pharmaceutical compositions.

15   [00394]   The precise dose to be employed in the formulation will also depend on the

route of administration, and the seriousness of the cancer, and should be decided according

to the judgment of the practitioner and each patient's circumstances. Effective doses may

be extrapolated from dose-response curves derived from *in vitro* or animal model test

systems.

20   [00395]   For EphA2 antigenic peptides or anti-idiotypic antibodies, the dosage

administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight.

Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the

patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight.

[00396]   With respect to the dosage of bacterial EphA2 vaccines of the invention, the

25   dosage is based on the amount colony forming units (c.f.u.). Generally, in various

embodiments, the dosage ranges are from about 1.0 c.f.u./kg to about 1×10^{10} c.f.u./kg;

from about 1.0 c.f.u./kg to about 1×10^8 c.f.u./kg; from about 1×10^2 c.f.u./kg to about 1 x

10^8 c.f.u./kg; and from about 1×10^4 c.f.u./kg to about 1×10^8 c.f.u./kg. Effective doses

may be extrapolated from dose-response curves derived animal model test systems. In

30   certain exemplary embodiments, the dosage ranges are 0.001-fold to 10,000-fold of the

murine LD_{50}, 0.01-fold to 1,000-fold of the murine LD_{50}, 0.1-fold to 500-fold of the murine

LD_{50}, 0.5-fold to 250-fold of the murine LD_{50}, 1-fold to 100-fold of the murine LD_{50}, and 5-

fold to 50-fold of the murine LD_{50}. In certain specific embodiments, the dosage ranges are

0.00.1-fold, 0.01-fold, 0.1-fold, 0.5-fold, 1-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold,

35   500-fold, 1,000-fold, 5,000-fold or 10,000-fold of the murine LD_{50}.

[00397]   For other cancer therapeutic agents administered to a patient, the typical

doses of various cancer therapeutics known in the art are provided in Table 5. Given the

invention, certain preferred embodiments will encompass the administration of lower

dosages in combination treatment regimens than dosages recommended for the

administration of single agents.

[00398]   The invention provides for any method of administrating lower doses of

known prophylactic or therapeutic agents than previously thought to be effective for the

prevention, treatment, management or amelioration of cancer. Preferably, lower doses of

known anti-cancer therapies are administered in combination with lower doses of EphA2

vaccines of the invention.

5.12 Kits

[00399]   The invention provides a pack or kit comprising one or more containers

filled with an EphA2 vaccine of the invention or a component of an EphA2 vaccine of the

invention. Additionally, one or more other prophylactic or therapeutic agents useful for the

treatment of a cancer or other hyperproliferative disorder can also be included in the pack or

kit. Optionally associated with such container(s) can be a notice in the form prescribed by a

governmental agency regulating the manufacture, use or sale of pharmaceuticals or

biological products, which notice reflects approval by the agency of manufacture, use or

sale for human administration.

[00400]   The present invention provides kits that can be used in the above methods.

In one embodiment, a kit comprises one or more a EphA2 vaccines of the invention. In

another embodiment, a kit further comprises one or more other prophylactic or therapeutic

agents useful for the treatment of cancer or another hyperproliferative disorder, in one or

more containers. In other embodiments, the prophylactic or therapeutic agent is a biological

or hormonal therapeutic.

6.   EXAMPLES: *LISTERIA*-BASED EPHA2 VACCINES PROVIDE THERAPEUTIC AND PROPHYLACTIC BENEFITS AGAINST EPHA2-EXPRESSING CANCERS

[00401]   The receptor tyrosine kinase EphA2 is selectively over-expressed in a variety

of malignant cell types and tumors. Additionally, recent studies have identified patient-

derived T lymphocytes that recognize EphA2. As such, EphA2 provides a much-needed

target for active immunotherapy. Here, we show that ectopic expression of human EphA2

in the Gram-positive facultative intracellular bacterium *Listeria monocytogenes* (*Listeria*)

can provide antigen-specific anti-tumor responses in vaccinated animals. *Listeria* infects

critical antigen presenting cells and thereby provides efficacy as a cancer therapy based its

ability to induce potent and robust CD4+ and CD8+ T cell responses against encoded

antigens. Attenuated *Listeria* mutant strains, which retain the antigen delivery potency of

wild-type bacteria, yet are nearly 10,000-fold less pathogenic in mice, were employed. To

demonstrate the efficacy of a Listeria-based EphA2 vaccine, *Listeria* actA⁻ strains were

5   engineered to express the extracellular (ECD) or intracellular (ICD) domain of human

EphA2 (actA-hEphA2-ECD or actA-hEphA2-ICD). Expression and secretion of hEphA2-

EX and -CO from *Listeria* was confirmed by Western blot analysis. Protective

immunization with actA-hEphA2EX significantly inhibited the subcutaneous growth of

CT26 cells that express full-length hEphA2 (p=0.0037). As controls, mice vaccinated with

10   the parental actA strain developed tumors that were comparable to vehicle-treated control

mice. Protective immunization with actA-hEphA2CO significantly increased the survival

rate in mice challenged with RenCA-hEphA2. Subsequently, the therapeutic efficacy of

actA-hEphA2-ECD or actA-hEphA2-ICD was evaluated using the experimental CT26-

hEphA2 lung tumor model. Following intravenous implantation of tumor cells, Balb/c mice

15   were immunized with actA, actA-hEphA2EX or actA-hEphA2-ICD. Immunization with

either actA-hEphA2-ECD or actA-hEphA2-ICD significantly prolonged survival (median

survival >43 days, p= 0.0035), as compared to matched controls (vehicle or actA median

survival time was 19 and 20 days, respectively). Importantly, 80% of the huEphA2

immunized mice survived until Day 43 following tumor implantation. Together, these data

20   demonstrate that Listeria-mediated vaccination targeting the EphA2 tumor antigen can

provide both preventative and therapeutic efficacy against a variety of malignancies.

6.1   EXAMPLE 1: *LISTERIA* LIFE CYCLE

[00402]   The life cycle of *Listeria monocytogenes*, encompassing the steps of

endocytosis, phagolysosomal lysis, and cell to cell spread, are shown in Figure 1A-1B.

25

6.2   EXAMPLE 2: CONSTRUCTION OF EphA2-EXPRESSING AND CONTROL *LISTERIA* STRAINS

6.2.1   Background

[00403]   Given the mechanisms by which *Listeria* programs the presentation of

30   heterologous antigens via the MHC class I pathway, the efficiency of both expression of

heterologous genes and secretion of the newly synthesized protein from the bacterium into

the cytoplasm of the infected (antigen presenting) cell is related directly to the potency of

CD8+ T cell priming and/or activation. As the level of Ag-specific T cell priming is related

directly to vaccine efficacy, the efficiency of heterologous protein expression and secretion

35   is linked directly to vaccine potency. Thus, the efficiency of EphA2 expression and

secretion was optimized to maximize the potency of *Listeria*-based vaccines, in terms of priming and/or activating CD8+ T cell responses specific for the encoded EphA2 protein.

6.2.2   Preparation of mutant *Listeria* strains.

[00404]   *Listeria* strains were derived from 10403S (Bishop *et al.*, *J. Immunol.*

5   139:2005 (1987)). *Listeria* strains with in-frame deletions of the indicated genes were generated by SOE-PCR and allelic exchange with established methods (Camilli *et al.*, *Mol. Microbiol.* 8:143 (1993)). The mutant strain LLO L461T (DP-L4017) was described in Glomski, *et al.*, *J. Cell. Biol.* 156: 1029 (2002), incorporated by reference herein. The *actA⁻* mutant (DP-L4029) is the DP-L3078 strain described in Skoble et al., *J. of Cell Biology*,

10   150: 527-537 (2000), incorporated by reference herein in its entirety, which has been cured of its prophage. (Prophage curing is described in (Lauer *et al.*, *J. Bacteriol.* 184:4177 (2002)); U.S. Patent Publication No. 2003/0203472.)

[00405]   In some vaccines, mutant strains of Listeria that are deficient with respect to internalin B (Genbank accession number AL591975 (*Listeria monocytogenes* strain EGD,

15   complete genome, segment 3/12; inlB gene region: nts. 97008-98963), incorporated by reference herein in its entirety, and/or the sequence listed as Genbank accession number NC_003210 (*Listeria monocytogenes* strain EGD, complete genome, *inlB* gene region: nts. 457008-458963), incorporated by reference herein in its entirety) are used. One particular *actA⁻inlB⁻* strain (DP-L4029*inlB*) was deposited with the American Type Culture Collection

20   (ATCC) on October 3, 2003, and designated with accession number PTA-5562).

6.2.3   Cloning vectors

[00406]   Selected heterologous antigen expression cassette molecular constructs were inserted into pPL2 (Lauer et. al. J. Bacteriol. 2002), or pAM401 (Wirth et. al., J. Bacteriol. 165:831-836), modified to contain the multiple cloning sequence of pPL2 (Aat II small

25   fragment, 171 bps), inserted between blunted *Xba I* and *Nru I* recognition sites, within the tetracycline resistance gene (pAM401-MCS). In general, the hly promoter and (selected) signal peptide sequence was inserted between the unique *Kpn I* and *Bam HI* sites in the pPL2 or pAM401-MCS plasmid vectors. Selected EphA2 genes (sometimes modified to contain N-terminal and C-terminal epitope tags; see description below) were cloned

30   subsequently into these constructs between unique Bam HI and Sac I sites. Molecular constructs based on the pAM401-MCS plasmid vector were introduced by electroporation into selected *Listeria* monocytogenes strains also treated with lysozyme, utilizing methods common to those skilled in the art. The expected plasmid structure in *Listeria*-transfectants was verified by isolating DNA from colonies that formed on chloramphenicol-containing

35   BHI agar plates (10 μg/ml) by restriction enzyme analysis. Recombinant *Listeria*

transformed with various pAM401-MCS based heterologous protein expression cassette constructs were utilized to measure heterologous protein expression and secretion, as described below.

[00407]   The pPL2 based heterologous protein expression cassette constructs were

5   incorporated into the tRNAArg gene in the genome of selected *Listeria* strains, according to the methods as described previously (Lauer *et al.*, 2002, *J. Bacteriol.* 184:4177-4186). Briefly, the pPL2 heterologous protein expression cassette constructs plasmid was first introduced into the E. coli host strain SM10 (Simon *et al.*, 1983, *Bio/Technology* 1:784-791) by electroporation or by chemical means. Subsequently, the pPL2-based plasmid was

10   transferred from transformed SM10 to the selected *Listeria* strains by conjugation. Following incubation on drug-selective BHI agar plates containing 7.5 µg of chloramphenicol per ml and 200 µg of streptomycin per ml as described, selected colonies are purified by passaging 3 times on plates with the same composition. To verify integration of the pPL2 vector at the phage attachment site, individual colonies are picked

15   and screened by PCR using the primer pair of forward primer NC16 (5'-gtcaaaacatacgctcttatc-3') (SEQ ID NO:47) and reverse primer PL95 (5'-acataatcagtccaaagtagatgc-3') (SEQ ID NO:48). Selected colonies having the pPL2-based plasmid incorporated into the tRNAArg gene in the genome of selected *Listeria* strains yielded a diagnostic DNA amplicon of 499 bps.

20   ### 6.2.4   Promoter

[00408]   Heterologous protein expression cassettes contained the prfA-dependent hly promoter, which drives the transcription of the gene encoding Listeriolysin O (LLO), and is activated within the microenvironment of the infected cell. Nucleotides 205586-206000 (414 bps) were amplified by PCR from *Listeria* monocytogenes, strain DP-L4056, using the

25   primer pair shown below. The region amplified includes the hly promoter and also the first 28 amino acids of LLO, comprising the secA1 signal peptide (ibid) and PEST domain. The expected sequence of this region for *Listeria* monocytogenes, strain EGD can be found in GenBank (Accession number: gi|16802048|ref|NC_003210.1|[16802048]).

[00409]   Primer Pair

30   [00410]   *Forward* (KpnI-LLO nts. 1257-1276):

[00411]   5'-CTCTGGTACCTCCTTTGATTAGTATATTC (SEQ ID NO:49)

[00412]   *Reverse* (Bam HI-LLO nts. X-x):

[00413]   5'-CTCTGGATCCATCCGCGTGTTTCTTTTCG (SEQ ID NO:50)

[00414]   (Restriction endonuclease recognition sites are underlined)

[00415]   The 422 bp PCR amplicon was cloned into the plasmid vector pCR-XL-
TOPO (Invitrogen, Carlsbad, CA), according to the manufacturer's specifications. The
nucleotide sequences of *Listeria*-specific bases in the pCR-XL-TOPO-hly promoter plasmid
clone was determined. *Listeria* monocytogenes strain DP-L4056 contained eight nucleotide

5   base changes flanking the prfA box in the hly promoter, as compared to the EGD strain.
The hly promoter alignment for the *Listeria* monocytogenes DP-L4056 and EGD strains is
shown in the Figure below (SEQ ID NOs: 68 and 69, respectively).

Listeria hly DP-L4056 and EGD Alignment

```
Query:        Listeria EGD
Subject:      DP-L4056 (wild-type, Portnoy strain)

                                                              prfA Box
Query: 1      ggtacctcctttgattagtatattcctatcttaaagtgacttttatgttgaggcattaac 60
              |||||||||||||||||||||||||||||||||||||||| |||||||||| |||||||||
Sbjct: 1      ggtacctcctttgattagtatattcctatcttaaagttacttttatgtggaggcattaac 60


Query: 61     atttgttaacgacgataaagggacagcaggactagaataaagctataaagcaagcatata 120
              |||||||||| |||| ||| ||| |||| ||||||||||||||||||||||||||||||||
Sbjct: 61     atttgttaatgacgtcaaaaggatagcaagactagaataaagctataaagcaagcatata 120


Query: 121    atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121    atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180

                                              Shine-Delgarno      LLO start
Query: 181    gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181    gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
```

10   [00416]   The 422 bp DNA corresponding to the hly promoter and secA1 LLO signal
peptide were liberated from the pCR-XL-TOPO-hly promoter plasmid clone by digestion
with *Kpn I* and *Bam HI*, and cloned into the pPL2 plasmid vector (Lauer *et al.*, 2002, *J.
Bact.*), according to conventional methods well-known to those skilled in the art. This
plasmid is known as pPL2-hlyP (native).

15   **6.2.5   Cloning and Insertion of EphA2 into pPL2 vectors for expression
                 in selected recombinant *Listeria monocytogenes* strains**

[00417]   The external (EX2) and cytoplasmic (CO) domains of EphA2 which flank
the EphA2 transmembrane helix were cloned separately for insertion into various pPL2-
signal peptide expression constructs. Genes corresponding to the native mammalian

20   sequence or codon-optimized for expression in *Listeria monocytogenes* of EphA2 EX2 and
CO domains were used. The optimal codons in *Listeria* (see table 3, *ibid*) for each of the 20
amino acids were utilized for codon-optimized EphA2 EX2 and EphA2 CO. The codon-
optimized EphA2 EX2 and CO domains were synthesized by extension of overlapping
oligonucleotides, using techniques common to those skilled in the art. The expected

25   sequence of all synthesized EphA2 constructs was verified by nucleotide sequencing.

[00418]   SEQ ID NOS:23, 21 and 22 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectively, for the EX2 domain of EphA2.

[00419]   SEQ ID NOS: 34, 32 and 33 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectivley, for the CO domain of EphA2.

[00420]   Additonally, FLAG (Stratagene, La Jolla, CA) and myc epitope tags were inserted, respectively, in-frame at the amino and carboxy termini of synthesized EphA2 EX2 and CO genes for detection of expressed and secreted EphA2 by Western blot analysis using antibodies specific for the FLAG or proteins. Thus, the expressed protein had the following ordered elements: NH_2-Signal Peptide-FLAG-EphA2-myc-CO_2. Shown below are the FLAG and myc epitope tag amino acid and codon-optimized nucleotide sequences.

[00421]   FLAG

[00422]   5'-GATTATAAAGATGATGATGATAAA (SEQ ID NO:51)

[00423] NH_2-DYKDDDDK-CO_2 (SEQ ID NO:52)

[00424]   Myc

[00425]   5'-GAACAAAAATTAATTAGTGAAGAAGATTTA (SEQ ID NO:53)

[00426] NH_2-EQKLISEEDL-CO_2 (SEQ ID NO:54)

6.2.6   Detection of synthesized and secreted heterologous proteins by Western blot analysis

[00427]   Synthesis of EphA2 protein and secretion from various selected recombinant *Listeria*-EphA2 strains was determined by Western blot analysis of trichloroacetic acid (TCA) precipitated bacterial culture fluids. Briefly, mid-log phase cultures of *Listeria* grown in BHI media were collected in a 50 mL conical centrifuge tube, the bacteria were pelleted, and ice-cold TCA was added to a final [6%] concentration to the bacterial culture supernatant and incubated on ice minimally for 90 min or overnight. The TCA-precipitated proteins were collected by centrifugation at 2400 X g for 20 min at 4°C. The pellet was then resuspended in 300-600 µl volume of TE, pH 8.0 containing 15 µg/ml phenol red. Sample dissolution was facilitated by vortexing. Sample pH was adjusted by NH_4OH addition if necessary until color was pink. All samples were prepared for electrophoresis by addition of 100 µl of 4X SDS loading buffer and incubating for 10 min. at 90°C. The samples were then centrifuged from 5 min at 14,000 rpm in a micro-centrifuge, and the supernatants collected and stored at –20°C. For Western bolt analysis, 20 µl of prepared fractions (the equivalent of culture fluids from of 1-4 x 10^9 bacteria), were loaded on the 4-12% SDS-PAGE gel, electrophoresed, and the proteins were transferred to PDDF

membrane, according to common methods used by those skilled in the art. Transferred
membranes were prepared s for incubation with antibody, by incubating in 5% dry milk in
PBS for 2 hr. at room temperature with agitation. Antibodies were used under the
following dilutions in PBST buffer (0.1% Tween 20 in PBS): (1) Rabbit anti-Myc
5   polyclonal antibody (ICL laboratories, Newberg, Oregon) at 1:10,000; (2) murine anti-
FLAG monoclonal antibody (Stratagene, *ibid*) at 1:2,000; and, (3) Rabbit anti-EphA2
(carboxy terminus-specific) polyclonal antibody (sc-924, Santa Cruz Biotechnology, Inc.,
Santa Cruz, CA). Specific binding of antibody to protein targets was evaluated by
secondary incubation with goat anti-rabbit or anti-mouse antibody conjugated with
10   horseradish peroxidase and detection with the ECL chemilumenescence assay kit
(Amersham), and exposure to film.

6.2.7   Secretion of EphA2 protein by recombinant *Listeria* encoding various forms of EphA2

6.2.7.1.   *Listeria*: [strains DP-L4029 (*actA*) or DP-L4017 (LLO
15   L461T)]

[00428]   Expression cassette construct: LLOss-PEST-CO-EphA2 (SEQ ID NO:35)

[00429]   The native sequence of the EphA2 CO domain was genetically fused to the
native secA1 LLO sequence, and the heterologous antigen expression cassette under control
of the *Listeria hly* promoter was inserted into the pPL2 plasmid between the *Kpn I* and *Sac I*
20   sites as described (*ibid*). The pPL2-EphA2 plasmid constructs were introduced by
conjugation into the *Listeria* strains DP-L4029 (actA) and DP-L4017 (L461T LLO) as
described (*ibid*). Figure 2 shows the results of a Western blot analysis of TCA-precipitated
bacterial culture fluids of 4029-EphA2 CO and 4017-EphA2 CO. This analysis
demonstrated that recombinant *Listeria* engineered to contain a heterologous protein
25   expression cassette comprised of native sequences corresponding to the secA1 and EphA2
CO fusion protein secreted multiple EphA2-specific fragments that were lower than the 52
kDa expected molecular weight, demonstrating the need for modification of the expression
cassette.

6.2.7.2.   *Listeria*: [DP-L4029 (*actA*)]

30   [00430]   Expression cassette constructs:
                  Native LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp (SEQ ID
                  NO:26)
                  (CodonOp) LLOss-PEST-(CodonOp)FLAG-EX2_EphA2-myc (SEQ ID
                  NO:28)

[00431]   The native secA1 LLO signal peptide sequence or secA1 LLO signal peptide

sequence codon-optimized for expression in *Listeria* was fused genetically with the EphA2

EX2 domain sequence codon-optimized for expression in *Listeria*, and the heterologous

antigen expression cassette under control of the *Listeria hly* promoter was inserted into the

5   pPL2 plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pPL2-EphA2

plasmid constructs were introduced by conjugation into the *Listeria* strain DP-L4029 (actA)

as described (*ibid*). Figure 3 shows the results of a Western blot analysis of TCA-

precipitated bacterial culture fluids of *Listeria* actA encoding either the native or codon-

optimized secA1 LLO signal peptide fused with the codon-optimized EphA2 EX2 domain.

10   This analysis demonstrated that the combination of utilizing sequence for both signal

peptide and heterologous protein optimized for the preferred codon usage in *Listeria*

monocytogenes resulted in expression of the expected full-length EphA2 EX2 domain

protein. Expression of full-length EphA2 EX2 domain protein was poor with codon-

optimization of the EphA2 coding sequence alone. The level of heterologous protein

15   expression (fragmented or full-length) was highest when utilizing the *Listeria*

monocytogenes LLO secA1 signal peptide, codon-optimized for expression in *Listeria*

monocytogenes.

6.2.7.3. *Listeria: [DP-L4029 (actA)]*

[00432]   Expression cassette constructs:

20   Native LLOss-PEST-(CodonOp) FLAG-EphA2_CO-myc (SEQ ID

                     NO:37)

                     CodonOp LLOss-PEST-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID

                     NO:39)

                     CodonOp PhoD-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID NO:41)

25   [00433]   The native secA1 LLO signal peptide sequence or the secA1 LLO signal

peptide sequence codon-optimized for expression in *Listeria*, or, alternatively, the Tat

signal peptide of the phoD gene from *Bacillus subtilis* codon-optimized for expression in

Listeria, was fused genetically with the EphA2 CO domain sequence codon-optimized for

expression in *Listeria*, and the heterologous antigen expression cassette under control of the

30   *Listeria hly* promoter was inserted into the pAM401-MCS plasmid between the *Kpn I* and

Sac I sites as described (*ibid*). The pAM401-EphA2 plasmid constructs were introduced by

electroporation into the *Listeria* strain DP-L4029 (actA) as described (*ibid*). Figure 4 shows

the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of *Listeria*

actA encoding either the native or codon-optimized secA1 LLO signal peptide, or codon-

35   optimized *Bacillus subtilis phoD* Tat signal peptide fused with the codon-optimized EphA2

CO domain. This analysis demonstrated once again that the combination of utilizing

sequence for both signal peptide and heterologous protein optimized for the preferred codon

usage in *Listeria monocytogenes* resulted in expression of the expected full-length EphA2

CO domain protein. Furthermore, expression and secretion of the expected full-length

5   EphA2 CO domain protein resulted from recombinant *Listeria* encoding codon-optimized

Bacillus subtilis phoD Tat signal peptide fused with the codon-optimized EphA2 CO

domain. This result demonstrates the novel and unexpected finding that signal peptides

from distinct bacterial species can be utilized to program the secretion of heterologous

proteins from recombinant *Listeria*. Expression of full-length EphA2 CO domain protein

10   was poor with codon-optimization of just the EphA2 sequence. The level of heterologous

protein expression was highest when utilizing signal peptides codon-optimized for

expression in *Listeria monocytogenes*.

6.2.8   Construction of *Listeria* strains expressing AH1/OVA or AH1-A5/OVA

15   [00434]   Mutant *Listeria* strains expressing a truncated form of a model antigen

ovalbumin (OVA), the immunodominant epitope from mouse colorectal cancer (CT26)

known as AH1 (SPSYVYHQF) (SEQ ID NO:55), and the altered epitope AH1-A5

(SPSYAYHQF (SEQ ID NO:56); Slansky *et al.*, 2000, *Immunity*, 13:529-538) were

prepared. The pPL2 integrational vector (Lauer *et al.*, *J. Bacteriol.* 184:4177 (2002); U.S.

20   Patent Publication No. 2003/0203472) was used to derive OVA and AH1-A5/OVA

recombinant *Listeria* strains containing a single copy integrated into an innocuous site of

the *Listeria* genome.

6.2.9   Construction of OVA-expressing *Listeria* (DP-L4056)

[00435]   An antigen expression cassette consisting of hemolysin-deleted LLO fused

25   with truncated OVA and contained in the pPL2 integration vector (pPL2/LLO-OVA) is first

prepared. The *Listeria*-OVA vaccine strain is derived by introducing pPL2/LLO-OVA into

the phage-cured *L. monocytogenes* strain DP-L4056 at the PSA (Phage from ScottA)

attachment site tRNA^{Arg}-*attBB'*.

[00436]   PCR is used to amplify the hemolysin-deleted LLO using the following

30   template and primers:

     Source: DP-L4056 genomic DNA

     Primers:

          Forward (*KpnI*-LLO nts. 1257-1276):

               5'-CTCTGGTACCTCCTTTGATTAGTATATTC (SEQ ID NO:57)

35   (T$_m$:LLO-spec: 52°C. Overall: 80°C.)

Reverse (*BamHI-XhoI*-LLO nts. 2811-2792):

> 5'-CAAT<u>GGATCCCTCGAG</u>ATCATAATTTACTTCATCCC
>
> (SEQ ID NO:58)
>
> (T_m:LLO-spec: 52°C. Overall: 102°C)

5 **[00437]**   PCR is also used to amplify the truncated OVA using the following template and primers:

> Source: pDP3616 plasmid DNA from DP-E3616 *E. coli* (Higgins et al., *Mol.*
>
> *Molbiol.* 31:1631-1641 (1999)).

> Primers:

10 > Forward (*XhoI- NcoI* OVA cDNA nts. 174-186):

> > 5'-ATTT<u>CTCGAG</u><u>CCATGG</u>GGGGGTTCTCATCATC
> >
> > (SEQ ID NO:59)
> >
> > (T_m: OVA-spec: 60°C. Overall: 88°C.)

> Reverse (*XhoI-NotI-HindIII*):

15 > > 5'-GGTG<u>CTCGAG</u><u>TGCGGCCGC</u>AAGCTT
> >
> > (SEQ ID NO:60)
> >
> > (T_m: Overall: 82°C.)

[00438]   One protocol for completing the construction process involves first cutting the LLO amplicon with *KpnI* and *BamHI* and inserting the *KpnI/BamHI* vector into the

20 pPL2 vector (pPL2-LLO). The OVA amplicon is then cut with *XhoI* and *NotI* and inserted into the pPL2-LLO which has been cut with *XhoI/NotI*. (Note: The pPL2 vector does not contain any *XhoI* sites; pDP-3616 contains one *XhoI* site, that is exploited in the OVA reverse primer design.) The construct pPL2/LLO-OVA is verified by restriction analysis (*KpnI*-LLO-*XhoI*-OVA-*NotI*) and sequencing. The plasmid pPL2/LLO-OVA is introduced

25 into *E. coli* by transformation, followed by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as described by Lauer et al. (or into another desired strain of *Listeria*).

6.2.10 <u>Construction of Listeria strains expressing AH1/OVA or AH1-A5/OVA</u>

30 **[00439]**   To prepare *Listeria* expressing either the AH1/OVA or the AH1-A5/OVA antigen sequences, inserts bearing the antigen are first prepared from oligonucleotides and then ligated into the vector pPL2-LLO-OVA (prepared as described above).

[00440]   The following oligonucleotides are used in preparation of the AH1 or AH1-A5 insert:

35 *AH1 epitope insert (ClaI-PstI compatible ends):*

Top strand oligo (AH1 Top):

5'-CGATTCCCCTAGTTATGTTTACCACCAATTTGCTGCA

(SEQ ID NO:61)

Bottom strand oligo (AH1 Bottom):

5   5'-GCAAATTGGTGGTAAACATAACTAGGGGAAT

(SEQ ID NO:62)

AH1-A5 epitope insert (ClaI-AvaII compatible ends):

[00441]   The sequence of the AH1-A5 epitope is SPSYAYHQF (SEQ ID NO:56) (5'-AGT CCA AGT Tat GCA Tat CAT CAA TTT-3') (SEQ ID NO:63).

10   Top: 5'-CGATAGTCCAAGTTATGCATATCATCAATTTGC

(SEQ ID NO:64)

Bottom: 5'-GTCGCAAATTGATGATATGCATAACTTGGACTAT

(SEQ ID NO:65)

[00442]   The oligonucletide pair for a given epitope are mixed together at an

15   equimolar ratio, heated at 95 °C for 5 min. The oligonucleotide mixture is then allowed to

slowly cool. The annealed oligonucleotide pairs are then ligated at a 200 to 1 molar ratio

with pPL2-LLO/OVA plasmid prepared by digestion with the relevant restriction enzymes.

The identity of the new construct can be verified by restriction analysis and/or sequencing.

[00443]   The plasmid can then be introduced into E. coli by transformation, followed

20   by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as

described by Lauer et al., or into another desired strain of *Listeria*, such as an *actA⁻* mutant

strain (DP-L0429), LLO L461T strain (DP-L4017), or *actA⁻/inlB⁻* strain (DP-L4029*inlB*).

6.3 EXAMPLE 3: GENERATION OF MURINE TUMOR CELL LINES THAT EXPRESS HUMAN EphA2

25   #### 6.3.1 Background

[00444]   A mouse immunotherapy model was created for testing the Listeria-based

vaccines of the invention. Three murine tumor cell lines, the CT26 murine colon carcinoma

cell line, the B16F10 murine melanoma cell line, and the RenCa murine renal cell

carcinoma cell line were created to express high levels of the huEphA2 protein. FACS cell

30   sorting assays were performed to identify CT26, B16F10, and RenCa tumor cells

expressing high levels of huEphA2, which were pooled and analyzed by Western blot

analysis. Clones were further pooled by FACS cell sorting to generate subclones

expressing the highest levels of huEphA2.

6.3.2 Selection of CT26 Murine Colon Carcinoma Cells Expressing
35   High Levels of huEphA2

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6.3.2.1. Transfection Assays With Lipofectamine™

[00445]   CT26 cells were transfected with constructs containing huEphA2 using standard transfection techniques and commercially available Lipofectamine™ according to the manufacturer's instructions.

6.3.2.2. Flow Cytometry (FACS) Analysis

[00446]   Single cell FACS sorting assays were performed by standard techniques to identify CT26 murine carcinoma tumor cell expressing high levels of human EphA2.

[00447]   Figure 5 illustrates a representative experiment, showing that the EphA2-3 clone expressed the highest levels of human EphA2 protein.

6.3.2.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00448]   Western blotting was also performed using standard techniques to determine the levels of human EphA2 protein expression in CT26 cells following FACS sorting of pooled populations of cells transfected with various constructs containing the huEphA2 gene. Figure 6 depicts results of a representative experiment. Compared to various clones tested, the huEphA2-3 clone expressed the highest levels of human EphA2 protein and was selected for the *in vivo* efficacy studies described below. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.3 Selection of B16F10 Murine Melanoma Cells Expressing High Levels of huEphA2

6.3.3.1. Retroviral Transduction

[00449]   Human EphA2 was introduced into B16F10 murine melanoma cells by a retroviral transduction method to create clones expressing high levels of the protein.

6.3.3.2. Flow Cytometry (FACS) Analysis

[00450]   As was performed on the CT26 cells, single cell FACS sorting assays were performed by standard techniques on B16F10 cells expressing huEphA2 to generate clones expressing high levels of huEphA2. Clones expressing the highest levels of huEphA2 were pooled and further examined by Western blot analysis. A representative FACS experiment is depicted in Figure 7, showing a B16F10 subclone expressing high levels of huEphA2.

6.3.3.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00451]   Western blotting was also performed as described above to determine levels of huEphA2 protein expression in B16F10 cells following FACS sorting of pooled

populations of cells containing the huEphA2 gene introduced by retroviral transduction. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.4   Selection of RenCa Murine Renal Cell Carcinoma Cells Expressing High Levels of huEphA2

6.3.4.1.   Transfection Assays With LipofectamineTM

[00452]   RenCa cells were transfected with constructs containing huEphA2 using standard transfection techniques and commercially available LipofectamineTM according to the manufacturer's instructions.

6.3.4.2.   Flow Cytometry (FACS) Analysis

[00453]   Single cell FACS sorting assays were performed by standard techniques to identify RenCa renal cell carcinoma tumor cells expressing high levels of human EphA2.

6.3.4.3.   Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00454]   Western blotting was also performed using standard techniques to determine the levels of human EphA2 protein expression in RenCa cells following FACS sorting of pooled populations of cells transfected with various constructs containing the huEphA2 gene. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.5   Transfection of 293 Cells with pCDNA4 plasmids encoding full-length EphA2

[00455]   Expression cassette constructs:

[00456]   pCDNA4-EphA2

[00457]   The native full-length EphA2 gene was cloned into the eukaryotic CMV promoter-based expression plasmid pCDNA4 (Invitrogen, Carlsbad, CA). Figure 8 shows the results of a Western blot analysis of lystates prepared from 293 cells transfected with the pCDNA4-EphA2 plasmid, and demonstrates the abundant expression in mammalian cells of full-length EphA2 protein.

6.4   EXAMPLE 4: Assessment of antigen-specific immune responses after vaccination.

[00458]   The vaccines of the present invention can be assessed using a variety of *in vitro* and *in vivo* methods. Some assays involve the analysis of antigen-specific T cells from the spleens of mice that have been vaccinated. For example C57Bl/6 or Balb/c are vaccinated by intravenous injection of 0.1 LD_{50} of a *Listeria* strain expressing OVA (or other appropriate antigen). Seven days after the vaccination, the spleen cells of the mice are

harvested (typically 3 mice per group) by placing the spleens into ice cooled RPMI 1640

medium and preparing a single cell suspension from this. As an alternative, the lymph

nodes of the mice could be similarly harvested, prepared as a single cell suspension and

substituted for the spleen cells in the assays described below. Typically, spleen cells are

5   assessed for intraveneous or intraperitoneal administration of the vaccine while spleen cells

and cells from lymph nodes are assessed for intramuscular, subcutaneous or intradermal

administration of the vaccine.

[00459]   Unless otherwise noted, all antibodies used in these examples can be

obtained from Pharmingen, San Diego, CA.

10   **6.4.1   ELISPOT Assay:**

[00460]   Using a *Listeria* strain having an OVA antigen as an example, the

quantitative frequency of antigen-specific T cells generated upon immunization in a mouse

model is assessed using an ELISPOT assay. The antigen-specific T cells evaluated are

OVA specific CD8+ or LLO specific CD8+ or CD4+ T cells. This OVA antigen model

15   assesses the immune response to a heterologous tumor antigen inserted into the vaccine and

could be substituted with any antigen of interest. The LLO antigen is specific to *Listeria*.

The specific T cells are assessed by detection of cytokine release (e.g. IFN-γ) upon

recognition of the specific antigen. PVDF-based 96 well plates (BD Biosciences, San Jose,

CA) are coated overnight at 4°C with an anti-murine IFN-γ monoclonal antibody (mAb R4;

20   5 μg/ml). The plates are washed and blocked for 2 hours at room temperature with 200 μL

of complete RPMI. Spleen cells from vaccinated mice (or non vaccinated control mice) are

added at 2×10^5 cells per well and incubated for 20 to 22 hours at 37°C in the presence of

various concentrations of peptides ranging from 0.01 to 10 μM. The peptides used for

OVA and LLO are either SL8, an MHC class I epitope for OVA, LLO_{190}

25   (NEKYAQAYPNVS, Invitrogen) an MHC class II epitope for listeriolysin O (*Listeria*

antigen), LLO_{296} (VAYGRQVYL), an MHC class I epitope for listeriolysin O, or LLO_{91}

(GYKDGNEYI), an MHC class I epitope for listeriolysin O. LLO_{190} and LLO_{296} are used

in a C57Bl/6 model, while LLO_{91} is used in a Balb/c model. After washing, the plates are

incubated with secondary biotinylated antibodies specific for IFN-γ (XMG1.2) diluted in

30   PBS to 0.5μg/ml. After incubation at room temperature for 2 hours, the plates are washed

and incubated for 1 hour at 37 °C with a 1 nm gold goat anti-biotin conjugate (GAB-1;

1:200 dilution; Ted Pella, Redding, CA) diluted in PBS containing 1 % BSA. After

thorough washing, the plates are incubated at room temperature for 2 to 10 minutes with

substrate (Silver Enhancing Kit; 30 ml/well; Ted Pella) for spot development. The plates are

35   then rinsed with distilled water to stop the substrate reaction. After the plates have been air-

dried, spots in each well are counted using an automated ELISPOT plate reader (CTL,

Cleveland, OH). The cytokine response is expressed as the number of IFN-γ spot-forming

cells (SFCs) per 2 x 10^5 spleen cells for either the OVA specific T cells or the *Listeria*

specific T cells.

5   **6.4.2   Intracellular Cytokine Staining Assay (ICS):**

[00461]   In order to further assess the number of antigen-specific CD8+ or CD4+ T

cells and correlate the results with those obtained from ELISPOT assays, ICS is performed

and the cells evaluated by flow cytometry analysis. Spleen cells from vaccinated and

control groups of mice are incubated with SL8 (stimulates OVA specific CD8+ cells) or

10   LLO$_{190}$ (stimulates LLO specific CD4+ cells) for 5 hours in the presence of Brefeldin A

(Pharmingen). The Brefeldin A inhibits secretion of the cytokines produced upon

stimulation of the T cells. Spleen cells incubated with an irrelevant MHC class I peptide are

used as controls. PMA (phorbol-12-myristate-13-acetate, Sigma) 20 ng/ml and ionomycin

(Sigma) 2 μg/ml stimulated spleen cells are used as a positive control for IFN-γ and TNF-α

15   intracellular cytokine staining. For detection of cytoplasmic cytokine expression, cells are

stained with FITC-anti-CD4 mAb (RM 4-5) and PerCP-anti-CD8 mAb (53-6.7), fixed and

permeabilized with Cytofix/CytoPerm solution (Pharmingen), and stained with PE-

conjugated anti-TNF-α mAb (MP6-XT22) and APC-conjugated anti-IFN-γ mAb (XMG1.2)

for 30 minutes on ice. The percentage of cells expressing intracellular IFN-γ and/or TNF-α

20   was determined by flow cytometry (FACScalibur, Becton Dickinson, Mountain View, CA)

and data analyzed using CELLQuest software (Becton Dickinson Immunocytometry

System). As the fluorescent labels on the various antibodies can all be distinguished by the

FACScalibur, the appropriate cells are identified by gating for those CD8+ and CD4+ that

are stained with either or both of the anti-IFN-γ or anti-TNF-α.

25   **6.4.3   Cytokine Expression of Stimulated Spleen Cells:**

[00462]   The level of cytokine secretion by the spleen cells of mice can also be

assessed for control and vaccinated C57Bl/6 mice. Spleen cells are stimulated for 24 hours

with SL8 or LLO$_{190}$. Stimulation with irrelevant peptide HSV-gB^2 (Invitrogen,

SSIEFARL) is used as a control. The supernatants of the stimulated cells are collected and

30   the levels of T helper-1 and T helper 2 cytokines are determined using an ELISA assay

(eBiosciences, CO) or a Cytometric Bead Array Kit (Pharmingen).

6.4.4   Assessment of Cytotoxic T cell Activity:

[00463]   The OVA specific CD8+ T cells can be further evaluated by assessing their

cytotoxic activity, either *in vitro* or directly in C57Bl/6 mouse *in vivo*. The CD8+ T cells

recognize and lyse their respective target cells in an antigen-specific manner. *In vitro* cytotoxicity is determined using a chromium release assay. Spleen cells of naïve and *Listeria*-OVA (internal) vaccinated mice are stimulated at a 10:1 ratio with either irradiated EG7.OVA cells (EL-4 tumor cell line transfected to express OVA, ATCC, Manassas, VA)

5   or with 100 nM SL8, in order to expand the OVA specific T cells in the spleen cell population. After 7 days of culture, the cytotoxic activity of the effector cells is determined in a standard 4-hour ^{51}Cr-release assay using EG7.OVA or SL8 pulsed EL-4 cells (ATCC, Manassas, VA) as target cells and EL-4 cells alone as negative control. The YAC-1 cell line (ATCC, Manassas, VA) is used as targets to determine NK cell activity, in order to

10   distinguish the activity due to T cells from that due to NK cells. The percentage of specific cytotoxicity is calculated as 100 x (experimental release – spontaneous release) / (maximal release – spontaneous release). Spontaneous release is determined by incubation of target cells without effector cells. Maximal release is determined by lysing cells with 0.1% Triton X-100. Experiments are considered valid for analysis if spontaneous release is < 20% of

15   maximal release.

[00464]   For the assessment of cytotoxic activity of OVA-specific CD8+ T cells *in vivo*, spleen cells from naïve C57Bl/6 mice are split into two equivalent aliquots. Each group is pulsed with a specific peptide, either target (SL8) or control (HSV-gB^2), at 0.5 μg/ml for 90 minutes at 37 °C. Cells are then washed 3 times in medium, and twice in PBS

20   + 0.1% BSA. Cells are resuspended at 1 x 10^7 per ml in warm PBS + 0.1% BSA (10 ml or less) for labeling with carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular Probes, Eugene, OR). To the target cell suspension, 1.25 μL of a 5mM stock of CFSE is added and the sample mixed by vortexing. To the control cell suspension, a ten-fold dilution of the CFSE stock is added and the sample mixed by vortexing. The cells are

25   incubated at 37 °C for 10 minutes. Staining is stopped by addition of a large volume (>40 ml) of ice-cold PBS. The cells are washed twice at room temperature with PBS, then resuspended and counted. Each cell suspension is diluted to 50 x 10^6 per ml, and 100 μL of each population is mixed and injected via the tail vein of either naïve or vaccinated mice. After 12-24 hours, the spleens are harvested and a total of 5 x 10^6 cells are analyzed by flow

30   cytometry. The high (target) and low (control) fluorescent peaks are enumerated, and the ratio of the two is used to establish the percentage of target cell lysis. The *in vivo* cytotoxicity assay permits the assessment of lytic activity of antigen-specific T cells without the need of *in vitro* re-stimulation. Furthermore, this assays assesses the T cell function in their native environment.

6.5 EXAMPLE 5: *IN VIVO* EphA2 EFFICACY STUDIES

6.5.1 Background

[00465]   Efficacy studies were performed in mice inoculated with CT26 tumor cells expressing the extracellular domain (ED) of human EphA2 in order to characterize the anti-
5   tumor effect of huEphA2. Endpoints measured were tumor volume and percent survival of the mice after tumor inoculation. The routes of inoculation were subcutaneous (s.c.) and intravenous (i.v.). HBSS and *Listeria* were administered as controls.

6.5.2 Control Vaccinations With AH1-A5-Expressing *Listeria*

[00466]   Balb/c mice (n=5) were immunized with 0.1 LD$_{50}$ *Listeria* 3 days post- i.v.
10   inoculation of 1 x 10^5 CT26 cells. Figure 9A demonstrates that therapeutic immunization with *Listeria* expressing AH1-A5 increases survival of the inoculated animals. Figure 9B shows the result of a separate but otherwise equivalent experiment in which lungs of the experimental mice were harvested on Day 19 following cell inoculation and fixed. Gross assessment of lung nodules was also performed, demonstrating the absence of tumors in the
15   lungs of test animals receiving *Listeria*-AH1/A5 as compared to control animals receiving a *Listeria* control.

6.5.3 Prophylactic EphA2 Vaccinations

6.5.3.1. Effect of Immunization with Listeria Expressing ECD of huEphA2 on CT26-hEphA2 Tumor Growth and
20   Survival

[00467]   Preventive studies were performed utilizing a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above. Groups of ten Balb/c mice per group were inoculated s.c. and groups of five mice per group were inoculated i.v. with CT26 colon carcinoma cells transfected with human EphA2 ("CT26-hEphA2"). The
25   mice were immunized with 0.1 LD50 *Listeria* control or *Listeria* expressing the ECD of hEphA2 in a 200μl bolus. For the studies entailing s.c. inoculations with CD26, AH1/A5 *Listeria* were used as a positive control. The immunizations were performed 14 and 4 days prior to CT26-hEphA2 tumor challenge. Tumor volume measurements were obtained twice weekly for the course of the study to determine an anti-tumor effect of the vaccinations.
30   [00468]   Figure 10A demonstrates the anti-tumor efficacy of *Listeria* expressing the ECD of hEphA2 against s.c. inoculations of huEphA2-expressing CT26 cells as compared to the negative controls (*p=0.0012). The data are summarized in Table 8 below:

Vaccination Group	Tumor Volume (mm^3 ± s.e.m.) (Day 42)	P vs. HBSS	P vs. *Listeria* Control

HBSS	1202.9 (± 321)	-	0.5528
Listeria Control	945.5 (± 338)	0.5528	-
Listeria-AH1/A5	392.5 (± 225)	0.0471	0.1895
Listeria-hEphA2-ECD	0.0 (± 0.0)	0.0012	0.0118

TABLE 8

[00469]   Figure 10B demonstrates the anti-tumor efficacy of *Listeria* expressing the
ECD of hEphA2 against i.v. inoculations of huEphA2-expressing CT26 cells as compared
to the negative controls *p=0.0017). The data are summarized in Table 9 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 65)
HBSS	18	-	0
Listeria Control	18	0.754	0
Listeria-AH1/A5	>65	0.0017	5
Listeria-hEphA2-ECD	>65	0.0017	3

TABLE 9

[00470]   Preventive studies were performed according to the schedule described
below. These studies utilized a pool of CT26 cells expressing huEphA2 generated by the
single cell FACS assays described above.

[00471]   Groups: Eight groups of ten mice per group. Groups 1-4 were inoculated
s.c. and groups 5-8 were inoculated i.v. with CT26 colon carcinoma cells transfected with
human EphA2, as shown in Table 10 below:

Treatment Group	Number of Mice per Groups
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. L4029 – AH1 *Listeria monocytogenes*	10
5. Control - HBSS	10
6. L4029 – control *Listeria monocytogenes*	10
7. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
8. L4029 – AH1 *Listeria monocytogenes*	10

TABLE 10

[00472]   Schedule: Animals received i.v. administrations of the agents listed above in
200µl bolus on Day 0 and Day 10. On Day 14, animals were inoculated with CT26 colon
carcinoma cells transfected with human EphA2 (L4029EphA2-exFlag), *Listeria* control
(L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) (5 x10⁵ cells
in 100µl volume) either subcutaneously or intravenously (experimental lung metastases
model). Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights

assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, *etc.*) were humanely euthanized. The experimental schedule is summarized in Table 11 below:

Group	Cell Inoculation Route (5 x10^5 cell in 100μl) (Day 14)	Primary Vaccination (Day 0)	Boost Vaccination (Day 10)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	2x10^7 CFU	2x10^7 CFU
3. L4029 EphA2-exFlag	s.c.	2x10^7 CFU	2x10^7 CFU
4. L4029 –AH1	s.c.	2x10^7 CFU	2x10^7 CFU
5. Control	i.v.	HBSS	HBSS
6. L4029	i.v.	2x10^7 CFU	2x10^7 CFU
7. L4029 EphA2-exFlag	i.v.	2x10^7 CFU	2x10^7 CFU
8. L4029 – AH1	i.v.	2x10^7 CFU	2x10^7 CFU

TABLE 11

[00473]   In this study, vaccination with *Listeria*-huEphA2 exFlag demonstrated a significant anti-tumor effect in both the s.c. and experimental lung metastases models (i.v.). In the s.c. model, a significant reduction in tumor growth was achieved with 3 mice remaining tumor-free. This response was also specific compared to the control *Listeria* and vehicle treated animals. In the experimental lung metastases model, vaccination with *Listeria* huEphA2-exFlag also demonstrated efficacy compared to the vehicle treated group.

[00474]   Figures 11A-11D illustrate results of the preventive experiments. Figure 11A shows that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 was significantly reduced when compared to vehicle (HBSS), *Listeria* (L4029) and *Listeria* positive (L4029-AH1) controls starting at day 21 and continued until day 32 post inoculation. Figure 11B also depicts results of the preventive experiments, showing again that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) was significantly reduced when compared to the *Listeria* (L4029) control starting at day 21 and continued until day 32 post inoculation. Figure 11C illustrates the results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate (indicated by triangles). Figure 11D illustrates the results of the prevention study in the lung metastases model, measuring the percent survival of the mice post tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate.

[00475]   The foregoing data demonstrate that preventative immunization with *Listeria* expressing the ECD of hEphA2 suppresses CT26-hEphA2 tumor growth and increases survival.

6.5.3.2. Effect of Immunization with Listeria Expressing ICD of huEphA2 on the Survival of Mice Inoculated with RenCa-hEphA2

[00476]   Preventive studies were performed utilizing a pool of RenCa cells (American Type Culture Collection, Manassas, VA) expressing huEphA2 generated and screened by the methods described above. Groups of ten Balb/c mice per group were inoculated subcutaneously with RenCa renal cell carcinoma cells expressing human EphA2 ("RenCa-hEphA2 cells"). The mice were immunized with 0.1 LD50 Listeria control or Listeria expressing the ICD of hEphA2 in a 200ml bolus. The immunizations were performed 18 and 4 days prior to RenCa-hEphA2 cell tumor challenge. Tumor volume measurements were obtained twice weekly for the course of the study to determine an anti-tumor effect of the vaccinations.

[00477]   Figure 12 demonstrates the anti-tumor efficacy of Listeria expressing the ICD of hEphA2 against s.c. inoculations of huEphA2-expressing RenCA cells as compared to the negative controls. A significant anti-tumor response, as assessed by increased survival via Kaplan-Meier analysis, was observed in animals vaccinated with Listeria expressing the ICD of hEphA2 as compared to animals vaccinated with Listeria alone (*p=0.0079).

6.5.4   Therapeutic EphA2 Vaccinations

[00478]   Therapeutic studies were performed utilizing a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above.

[00479]   A representative therapeutic study was performed as follows:

[00480]   Groups: Six groups of ten mice per group. Groups 1-3 were inoculated s.c. and groups 4-6 were inoculated i.v. with CT26 murine colon carcinoma cells, as shown in Table 12 below:

Treatment Group	Number of Mice per Groups
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. Control - HBSS	10
5. L4029 – control *Listeria monocytogenes*	10
6. L4029-EphA2 exFlag – *Listeria monocytogenes*	10

expressing extracellular domain of human EphA2	

TABLE 12

[00481]   Schedule: Animals were inoculated with CT26 colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), *Listeria* control (L4029-control) or vehicle (HBSS) (5×10^5 cells in 100ml volume) either subcutaneously or intravenously (experimental lung metastases model). Three days after cell inoculation, animals received i.v. administrations of the agents listed above in 200ml bolus. Two weeks following the first administration, the animals received a booster vaccination. Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, etc.) were humanely euthanized. The schedule is summarized in Table 13 below.

Group	Cell Inoculation Route (5×10^5 cell in 100μl)	Primary Vaccination (Day 3)	Boost Vaccination (Day 17)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
3. L4029 EphA2-exFlag	s.c.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
4. Control	i.v.	HBSS	HBSS
5. L4029	i.v.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
6. L4029 EphA2-exFlag	i.v.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU

TABLE 13

[00482]   Figures 13A-13C illustrate the results of a typical therapeutic study. In Figure 13A, tumor volume was measured at several intervals post inoculation. Compared to the HBSS and Listeria controls, the mice inoculated with CT26 cells expressing the ECD of huEphA2 had a significantly lower tumor volume after day 14 and continued onto day 28. Figure 13B depicts the mean tumor volume of mice inoculated with CT26 cells containing either Listeria control or huEphA2. Compared to control, the mice inoculated with CT26 cells expressing huEphA2 had a reduced mean tumor volume. Figure 13C represents the results of the therapeutic study using the lung metastases model, measuring percent survival of the mice post inoculation with CT26 cells with either HBSS or Listeria control, or Listeria expressing the ECD of huEphA2. Animals inoculated with CT26 cells expressing the ECD of huEphA2 (depicted by triangles) showed a higher percent survival rate compared to controls.

[00483]   In another study, groups of ten Balb/c mice per group were inoculated s.c. or

i.v. with CT26 colon carcinoma cells transfected with human EphA2 ("CT26-hEphA2").

The mice were immunized with 0.1 LD$_{50}$ actA *Listeria* control or *Listeria* expressing the

ICD of hEphA2 in a 200µl bolus. In one regimen, the immunizations were performed 6 and

5   14 days post s.c. CT26-hEphA2 tumor inoculation. In another regimen, the immunizations

were performed 3 and 14 days post i.v. CT26-hEphA2 tumor inoculation. Anti-tumor

efficacy was determined from twice weekly tumor measurements and survival.

[00484]   Significant anti-tumor efficacy was observed in the *Listeria*-hEphA2

vaccinated animals (p=0.0035).

10   [00485]   Figure 14A demonstrates the tumor measurements of immunized animals.

This data is summarized in Table 14 below:

Vaccination Group	Tumor Volume (mm^3 ± s.e.m.) (Day 21)	P vs. HBSS	P vs. *Listeria* Control
HBSS	1827 (± 518)	-	0.961
Listeria Control	1799 (± 267)	0.961	-
Listeria-AH1/A5	0	0.0005	0.000003
Listeria-hEphA2-ICD-1	694 (± 232)	0.0054	0.006
Listeria-hEphA2-ICD-2	731 (± 176)	0.052	0.004

TABLE 14

[00486]   Figure 14B demonstrates the survival time of immunized animals. This data

15   is summarized in Table 15 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 65)
HBSS	19	-	0
Listeria Control	20	Ns	0
Listeria-hEphA2-ICD-1	>65	0.0035	3
Listeria-hEphA2-ICD-2	>65	0.0035	4
Listeria-hEphA2-ICD-3	>65	0.0035	4

TABLE 15

[00487]   Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors

with recombinant *Listeria* encoding OVA.AH1 (MMTV gp70 immunodominant epitope) or

20   OVA.AH1-A5 (MMTV gp70 immunodominant epitope, with heteroclitic change for

enhanced T-cell receptor binding) confers long-term survival (Figure 14C).

[00488]   The EphA2 CO domain is strongly immunogenic, and a significant long term

increase in survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was

observed when immunized with recombinant *Listeria* encoding codon-optimized or native

25   EphA2 CO domain sequence (Figure 14D).

[00489]   The EphA2 EX2 domain is poorly immunogenic, and increased survival of
Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was observed only when
immunized with recombinant *Listeria* encoding codon-optimized secA1 signal peptide
fused with the codon-optimized EphA2 EX2 domain sequence. Therapeutic efficacy was
5   not observed in mice when immunized with recombinant *Listeria* encoding native secA1
signal peptide fused with the codon-optimized EphA2 EX2 domain sequence (Figure 14E).
The desirability of using both codon-optimized secA1 signal peptide and EphA2 EX2
domain sequences was supported by statistically significant therapeutic anti-tumor efficacy,
as shown in the table below:

10   [00490]   A comparison by log-rank test of survival curves shown in Figure 14E and
summarized in Table 16 below:

Experimental Group	Median Survival (Days)	Significance versus HBSS cohort (p value)	Significance versus actA-native secA1/EphA2 EX2 cohort (p value)
HBSS	19	-	-
ActA	20	NS	NS
actA-native secA1-EphA2 EX2 (native)	19	NS	-
actA-native secA1-EphA2 EX2 (CodOp)	24	0.0035	NS
actA-CodOp secA1-EphA2 EX2 (CodOp)	37	0.0035	0.0162
actA-native secA1-EphA2 CO (CodOp)	>99	0.0035	0.0015

TABLE 16

[00491]   Significantly, even though pCDNA4-EphA2 plasmid transfected 293 cells
15   yielded very high levels of protein expression, immunization of Balb/C mice bearing
CT26.24 (huEphA2+) lung tumors with the pCDNA4-EphA2 plasmid did not result in any
observance of therapeutic anti-tumor efficacy (Figure 14F).

[00492]   For therapeutic *in vivo* tumor studies, female Balb/C mice were implanted IV
with 5 x 10^5 CT26 cells stably expressing EphA2. Three days later, mice were randomized
20   and vaccinated IV with various recombinant *Listeria* strains encoding EphA2. In some
cases (noted in figures) mice were vaccinated with 100 µg of pCDNA4 plasmid or
pCDNA4-EphA2 plasmid in the *tibialis* anterior muscle. As a positive control, mice were
vaccinated IV with recombinant *Listeria* strains encoding OVA.AHI or OVA.AH1-A5
protein chimeras. Mice were vaccinated on days 3 and 14 following tumor cell
25   implantation. Mice injected with Hanks Balanced Salt Solution (HBSS) buffer or
unmodified *Listeria* served as negative controls. All experimental cohorts contained 5

mice. For survival studies mice were sacrificed when they started to show any signs of stress or labored breathing.

[00493]   The foregoing data demonstrate that therapeutic immunization with *Listeria* expressing the hEphA2 suppresses established CT26-hEphA2 tumor growth and increases

5   survival.

6.6 EXAMPLE 6: Long-term Suppression of CT26-hEphA2 Tumor Growth Upon Rechallenge

[00494]   Balb/c mice failing to form tumors after preventative immunization with *Listeria* expressing either the ICD or ECD of hEphA2 against CT26-hEphA2 tumor

10   challenged, were re-challenged (s.c.) with both CT26 parental cell line and CT26-hEphA2 cells on opposite flanks 56 days after initial tumor challenge and 60 days after the last immunization. Age-matched mice were used as a control in this experiment.

[00495]   Re-challenge with parental CT26 cells showed no statistically significant differences in tumor growth between groups (data not shown). However, as shown in

15   Figure 15, both groups vaccinated with *Listeria* expressing either the ICD or ECD of hEphA2 demonstrated a significant suppression of tumor growth upon re-challenge ($*p < 0.041$).

6.7 EXAMPLE 7: Immunization with Listeria Expressing hEphA2 Elicits an EphA2-Specific CD8+ T Cell Response.

20   [00496]   Balb/c mice (n=3) were immunized with *Listeria* L461T expressing the intracellular domain of hEphA2 (hEphA2-ICD) or ΔactA expressing codon optimized extracellular domain of hEphA2 (hEphA2-ECD) two weeks apart. Mice were euthanized, and spleens harvested and pooled 6 days after the last immunization. For the ELISPOT assay, the cells were re-stimulated *in vitro* with P815 cells expressing full-length hEphA2

25   or cell lysates prepared from these cells. The parental P815 cells or cell lysates served as a negative control. Cells were also stimulated with recombinant hEphA2 Fc fusion protein. IFN-gamma positive spot forming colonies (SFCs) were measured using a 96 well spot reader.

[00497]   As shown in Figure 16, increased IFN-gamma SFCs were observed with

30   spleen cells derived from mice vaccinated with Listeria-hEphA2. Both hEphA2 expressing cells or cell lysates stimulation resulted in an increase in IFN-gamma SFC which suggests an EphA2-specific CD8+ as well as CD4+ T cell response. Spleen cells from mice vaccinated with the parental *Listeria* control did not demonstrate an increase in IFN-gamma SFC.

6.8 EXAMPLE 8: Both CD4+ and CD8+ T Cell Responses are Required for Maximal hEphA2-Directed Anti-Tumor Efficacy.

[00498] Balb/c mice (n=10) were inoculated i.v. with 2 x 10^5 CT26-hEphA2 on day 0. CD4+ cells and CD8+ T-cells were depleted by injecting 200 µg anti-CD4 (ATCC hybridoma GK1.5) or anti-CD8 (ATCC hybridoma 2.4-3) on Days 1 and 3, which was confirmed by FACS analysis (data not shown). Mice were then immunized i.v. with 0.1 LD_{50} Listeria L461T expressing hEphA2 ICD on Day 4 and monitored for survival.

[00499] As shown in Figure 17, both CD4+ and CD8+ depleted groups failed to demonstrate the degree of anti-tumor response seen in the non-T cell depleted animals. The data are summarized in Table 17 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 67)
HBSS	17	-	0
Listeria-hEphA2-ICD	>67	<0.0001	7
Listeria-hEphA2-ICD + anti-CD4	19	0.03	2
Listeria-hEphA2-ICD + anti-CD8	24	0.0002	0

TABLE 17

[00500] The foregoing data indicate a requirement for both CD4+ and CD8+ T cells in optimal suppression of tumor growth.

6.9 EXAMPLE 9: Therapeutic Vaccination with Listeria Expressing Human EphA2 ICD Enhances CD45+ Tumor Infiltrate

[00501] Balb/c mice (n=3) were immunized with 0.1 LD50 actA-Listeria control or Listeria expressing either the ECD or ICD of hEphA2, 6 days post s.c. CT26-hEphA2 tumor inoculation. 9 days post-vaccination, tumors were harvested, fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 4µm. Microscope slides were prepared from the tumor sections. The tissues on the slides were deparaffinized and rehydrated as follows: 4 changes with xylene, 5 minutes each; 2 changes with absolute alcohol, 5 minutes each; 1 change with 95% alcohol for 5 minutes; 1 change with 70% for 5 minutes; and two changes with distilled water.

[00502] Steam antigen retrieval was performed in a Black and Decker Rice steamer using target antigen retrieval (TAR) solution (DakoCytomation, Carpinteria, CA) using a modification of the manufacture's protocol. The slides were placed into TAR solution preheated to just below boiling temperature and incubated for 20 minutes. The slides were then removed from the TAR solution and allowed to cool at room temperature for 20 minutes, and rinsed twice in TBS assay buffer.

[00503]   Staining of the slides with biotinylated antibody was performed as follows.

[00504]   Endogenous peroxidase was blocked by immersing the slides in solution of 3% hydrogen peroxide in methanol, for 10 minutes, followed with 2 changes of distilled water, 5 minutes each. Protein was blocked by immersing the slides in a solution of 5% Bovine Serum Albumin (BSA) in 1x Tris buffered saline with 0.01% Tween 20 (TBST) for at least 30 minutes.

[00505]   After wiping excess BSA solution from the slide, creating a "pool", centered around tissue, the slide was laid flat in humid chamber and biotinylated rat anti-mouse CD45/B220 (Pharmingen) at 1:100 dilution in a solution of 1%BSA/TBST was applied. The slide was incubated in a humid chamber overnight at room temperature with care taken to prevent drying of the tissue sections.

[00506]   The next morning, the slides are washed with 2 changes of TBST, the second one lasting 10 minutes. Streptavidin conjugated with either HRP or AP is applied, incubating for 30 minutes at room temp. The slides are washed with two changes of TBST, visualized with an appropriate substrate chromagen (for Strep-HRP, DAB is used). After a wash in distilled water, the slides are counterstained with Mayers Hematoxylin by immersing the slides in dye for 2 minutes. The slides are then washed in running tap water until water runs clear, immersed in bluing agent (Scotts substitute tap water) for 30 seconds, and washed again in tap water. The slides are dehydrated and cleared in graded alcohols through xylene (or xylene substitute) by the following washes: 95% alcohol for 1 minute, 3 changes absolute alcohol for 1 minute each, and 4 changes xylenes for 1 minute each.

[00507]   Mounting media is applied to the cover slips (for xylene, DPX mountant is used) and the slides are allowed to dry over night prior to visualization.

[00508]   The sections were visualized on a Nikon Eclipse E400 and images captured with a Nikon DXM1200 digital camera (Figure 18A). Data was further normalized to tumor volume (Figure 18B).

[00509]   The results demonstrate that tumor associated infiltrating lymphocytes are increased following therapeutic vaccination.

7. EQUIVALENTS

[00510]   Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00511]   All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent

as if each individual publication, patent or patent application was specifically and

individually indicated to be incorporated herein by reference.

We claim:

1.   A method of eliciting an immune response against an EphA2-expressing cell, said
method comprising administering to an individual a composition comprising an EphA2
antigenic peptide in an amount effective to elicit an immune response against an EphA2-

5   expressing cell.

2.   The method of claim 1, wherein the EphA2 antigenic peptide is not TLADFDPRV
(SEQ ID NO:3); VLLLVLAGV (SEQ ID NO:4); VLAGVGFFI (SEQ ID NO:5);
IMNDMPIYM (SEQ ID NO:6); SLLGLKDQV (SEQ ID NO:7); WLVPIGQCL (SEQ ID
NO:8); LLWGCALAA (SEQ ID NO:9); GLTRTSVTV (SEQ ID NO:10); NLYYAESDL

10   (SEQ ID NO:11); KLNVEERSV (SEQ ID NO:12); IMGQFSHHN (SEQ ID NO:13);
YSVCNVMSG (SEQ ID NO:14); MQNIMNDMP (SEQ ID NO:15);
EAGIMGQFSHHNIIR (SEQ ID NO:16); PIYMYSVCNVMSG (SEQ ID NO:17); or
DLMQNIMNDMPIYMYS (SEQ ID NO:18).

3.   The method of claim 1, wherein the composition further comprises an adjuvant.

15   4.   The method of claim 1, wherein the composition comprises a heat shock protein bound
to said EphA2 antigenic peptide.

5.   The method of claim 1, where the polypeptide further comprises a protein transduction
domain.

6.   The method of claim 5, wherein the protein transduction domain is the Antennapedia or

20   the HIV tat protein transduction domain.

7.   A method of eliciting an immune response against an EphA2-expressing cell, said
method comprising administering to an individual a composition comprising an EphA2
antigenic peptide expression vehicle in an amount effective to elicit an immune response
against an EphA2-expressing cell.

25   8.   The method of claim 7, wherein the expression vehicle is a nucleic acid encoding said
EphA2 antigenic peptide operably linked to a promoter.

9. The method of claim 8, wherein the nucleic acid is DNA.

10. The method of claim 9, wherein the DNA is conjugated to a carrier.

11. The method of claim 8, wherein the carrier is asialoglycoprotein.

12. The method of claim 8, wherein the carrier is transferrin.

13. The method of claim 8, wherein the carrier is polymeric IgA.

14. The method of claim 7, wherein the expression vehicle is an infectious agent comprising a nucleic acid, said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic peptide operably linked to a promoter.

15. The method of claim 14, wherein the sequence encoding said EphA2 antigenic peptide is codon-optimized for expression in said infectious agent.

16. The method of claim 14, wherein the infectious agent is coated with a reagent that targets the infectious agent to EphA2-expressing cells.

17. The method of claim 16, wherein the reagent is an anti-EphA2 antibody.

18. The method of claim 14, wherein the infectious agent is coated with a reagent that targets the infectious agent to antigen-presenting cells.

19. The method of claim 14, wherein the infectious agent is a bacterium.

20. The method of claim 19, wherein the bacterium is attenuated.

21. The method of claim 19, wherein the nucleic acid comprises a nucleotide sequence encoding a secretory signal operatively linked to the sequence encoding the EphA2 antigenic peptide.

22. The method of claim 21, wherein the secretory signal is a SecA secretory signal.

23. The method of claim 19, wherein the bacterium is *Pseudomonas aeruginosa*.

24. The method of claim 19, wherein the bacterium is not *Listeria*.

25. The method of claim 14, wherein the infectious agent is a virus.

26. The method of claim 25, wherein the virus is a retrovirus.

5   27. The method of claim 26, wherein the retrovirus is a lentivirus.

28. The method of claim 25, wherein the virus is an adenovirus.

29. The method of claim 25, wherein the virus is an adeno-associated virus.

30. The method of claim 25, wherein the virus is herpes simplex virus.

31. The method of claim 25, wherein the virus is attenuated.

10   32. The method of claim 7, wherein the expression vehicle is a mammalian cell comprising
a recombinant nucleic acid, said nucleic acid comprising a nucleotide sequence encoding
said EphA2 antigenic peptide.

33. The method of claim 33, wherein the mammalian cell is a human cell.

34. The method of claim 32, wherein the mammalian cell is encapsulated within a
15   membrane.

35. The method of claim 34, wherein said administering is by means of implantation.

36. A method of eliciting an immune response against an EphA2-expressing cell, said
method comprising administering to an individual a composition comprising antigen
presenting cells sensitized with an EphA2 antigenic peptide.

20   37. The method of claim 36, further comprising prior to said administration the step of
sensitizing the antigen presenting cells.

38. The method of claim 37, wherein the antigen presenting cells are sensitized by a method comprising: contacting the cells with a composition comprising one or more EphA2 antigenic peptides in an amount effective to sensitize the cells.

39. The method of claim 36, wherein the composition further comprises a heat shock
5   protein.

40. The method of claim 39, wherein the heat shock protein is hsp70, gp96, or hsp90.

41. The method of claim 36, wherein the antigen presenting cells are autologous to the individual.

42. The method of claim 36, wherein the antigen presenting cells are non-autologous to the
10   individual.

43. The method of claim 36, wherein the antigen presenting cells are macrophages.

44. The method of claim 36, wherein the antigen presenting cells are dendritic cells.

45. The method of claim 1, 7, or 36, wherein the individual has cancer.

46. The method of claim 45, wherein said cancer is of an epithelial cell origin or
15   endothelial cell origin.

47. The method of claim 45, wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

48. The method of claim 45, wherein said cancer is a cancer of the skin, lung, colon, ovary, esophagus, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

20   49. The method of claim 1, 7, 36, or 52, wherein the individual has a non-neoplastic hyperproliferative disorder.

50. The method of claim 49, wherein the hyperproliferative disorder is an epithelial cell disorder.

51. The method of claim 50, wherein the hyperproliferative is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

52. A method of eliciting an immune response against an EphA2-expressing cell, said
5   method comprising administering to an individual a composition comprising an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody in an amount effective to elicit an immune response against an EphA2-expressing cell.

53. A method of treating a human individual having a hyperproliferative disorder of
10   EphA2-expressing cells, said method comprising administering to the individual a composition comprising an EphA2 antigenic peptide in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

54. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a
15   composition comprising an EphA2 expression vehicle in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

55. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a composition comprising antigen presenting cells sensitized with an EphA2 antigenic
20   peptide in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

56. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to an individual a composition comprising an anti-idiotypic antibody or antigen-binding fragment thereof
25   which immunospecifically binds to an idiotype of an anti-EphA2 antibody in an amount effective to elicit treat a hyperproliferative disorder of EphA2-expressing cells.

57. The method of claim 53, 54, 55, or 56, wherein the individual has cancer.

58. The method of claim 57, wherein said cancer is of an epithelial cell origin.

59. The method of claim 57, wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

60. The method of claim 57, wherein said cancer is a cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

61. The method of claim 53, 54, 55, or 56, wherein the individual has a non-neoplastic hyperproliferative disorder.

62. The method of claim 61, wherein the hyperproliferative disorder is an epithelial cell disorder.

63. The method of claim 62, wherein the hyperproliferative disorder is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

64. The method of any one of claims 1, 7, 36, 53, 54, and 55, wherein the EphA2 polypeptide comprises full length EphA2.

65. The method of any one of claims 1, 7, 36, 53, 54, and 55, wherein the EphA2 polypeptide comprises the extracellular domain or intracellular domain of EphA2.

66. The method of any one of claims 1, 7, 36, 53, 54, and 55, wherein the EphA2 polypeptide comprises the intracellular domain and extracellular domain of EphA2 and lacks the transmembrane domain.

67. The method of claim 66, wherein the EphA2 polypeptide lacks tyrosine kinase activity.

68. The method of claim 67, wherein the EphA2 polypeptide lacks tyrosine kinase activity due to a lysine to methionine substitution at position 646 of EphA2.

69. The method of any one of claims 1, 7, 36, 53, 54, and 55, wherein the EphA2 polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

70. The method of claim 52 or 56, wherein the EphA2 antibody immunospecifically binds to an epitope in the extracellular domain of EphA2.

71. The method of claim 52 or 56, wherein the EphA2 antibody immunospecifically binds to an epitope the intracellular domain of EphA2.

72. The method of any one of claims 1, 7, 36, 53, 54, and 55, wherein the EphA2 polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

73. The method of claim 1, or 53, wherein the composition comprises a plurality of EphA2 antigenic peptides.

74. The method of claim 7 or 54, wherein the composition comprises a plurality of EphA2 antigenic peptide expression vehicles.

75. The method of claim 7 or 54, wherein the expression vehicle expresses a plurality of EphA2 antigenic peptides.

76. The method of claim 36 or 55, wherein the antigen presenting cells are sensitized with a plurality of EphA2 antigenic peptides

77. The method of any one of claims 1, 7, 36, 52, 53, 54, 55, and 56, further comprising administering an additional anti-cancer therapy.

78. The method of claim 77, wherein the additional anti-cancer therapy is an agonistic EphA2 antibody.

79. The method of claim 77, wherein the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

80. The method of any one of claims 1, 7, 36, 52, 53, 54, 55, and 56, wherein said administering is mucosal, intranasal, parenteral, intramuscular, intravenous, oral or intraperitoneal.

81. The method of any one of claims 1, 7, 36, 52, 53, 54, 55, and 56, wherein the
5   administration elicits a CD4$^+$ T-cell response, a CD8$^+$ T-cell response, an innate immune response, an antibody response, or a combination of one or more of the foregoing.

82. The method of claim 81, wherein the administration elicits both a CD4$^+$ T-cell response and a CD8$^+$ T-cell response.

83. The method of claim 1, 7, 36 or 54, wherein the subject has a disease involving
10   aberrant angiogenesis.

84. The method of claim 83, wherein the wherein the disease is macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus,
15   psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis or coronary artery disease.

85. A method of treating a human individual having a disease involving aberrant angiogenesis, said method comprising administering to the individual a composition comprising an EphA2 antigenic peptide in an amount effective to treat a disease involving
20   aberrant angiogenesis.

86. A method of treating a human individual having a a disease involving aberrant angiogenesis, said method comprising administering to the individual a composition comprising an EphA2 expression vehicle in an amount effective to treat a disease involving aberrant angiogenesis.

25   87. A method of treating a human individual having a disease involving aberrant angiogenesis, said method comprising administering to the individual a composition

comprising antigen presenting cells sensitized with an EphA2 antigenic peptide in an amount effective to treat a disease involving aberrant angiogenesis.

88. A method of treating a human individual having a a disease involving aberrant angiogenesis, said method comprising administering to an individual a composition

5 comprising an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody in an amount effective to elicit treat a disease involving aberrant angiogenesis.

89. The method of claim 85, 86, 87, or 88, wherein the wherein the disease is macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile

10 hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis or coronary artery disease.

90. A method of producing antibodies that immunospecifically bind to EphA2 comprising

15 administering an EphA2 vaccine to a host.

91. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a composition comprising antibodies produced by administering an EphA2 vaccine to a host in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

20 92. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising (1) administering to the individual a composition comprising an EphA2 expression vehicle, selected from the group consisting of a bacterium or virus, in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells; and (2) administering to the individual an antibiotic or antiviral

25 agent in an amount effective to treat a bacterial or viral infection.
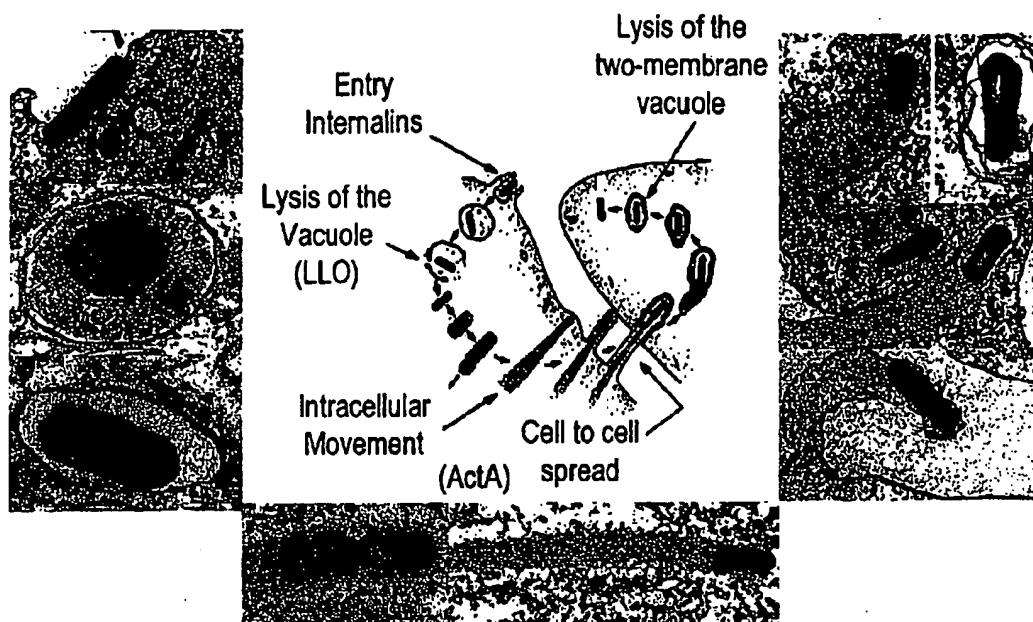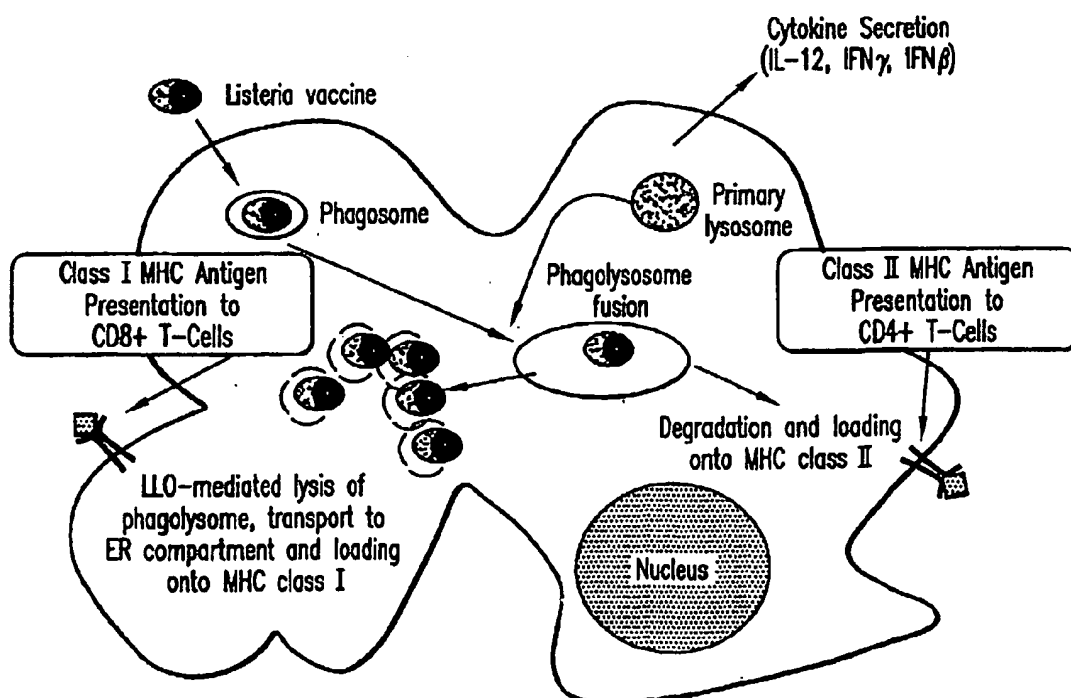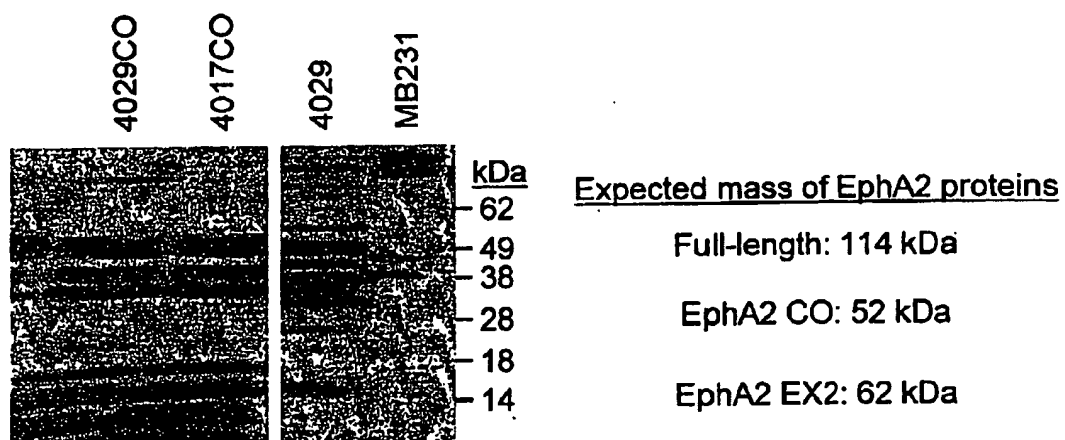
FIG.1A

FIG.1B

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Expected mass of EphA2 proteins

Full-length: 114 kDa

EphA2 CO: 52 kDa

EphA2 EX2: 62 kDa

Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

FIG.2

Codon-Optimized Antigen: hEphA2
                                EX2

Codon-Optimized secA1:   -   +   act A

kDa

Expected mass of EphA2 proteins   62
Full-length: 114 kDa
EphA2 CO: 52 kDa   49
EphA2 EX2: 62 kDa   38

                                         28

                                         18

Ab: Rabbit anti-myc polyelonal antibody

FIG.3

FIG.4

Ab: Rabbit anti-myc polyclonal antibody
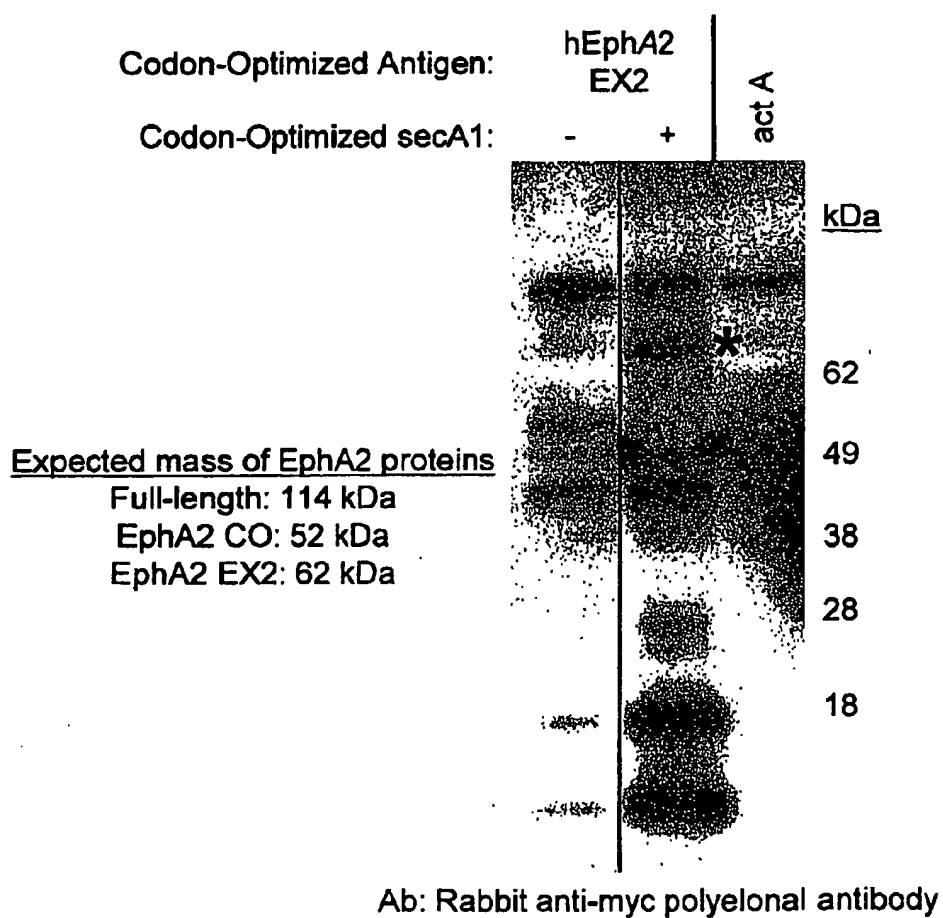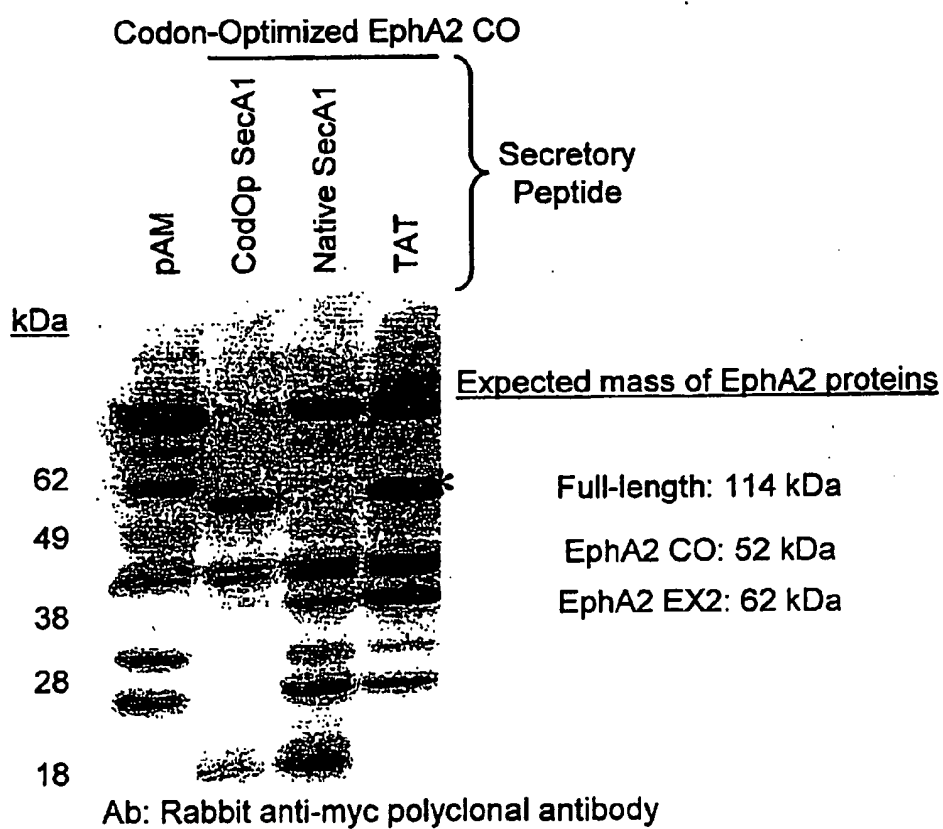
Human EphA2 Expression in CT26 Subclones
Generation of "the Super Clone"
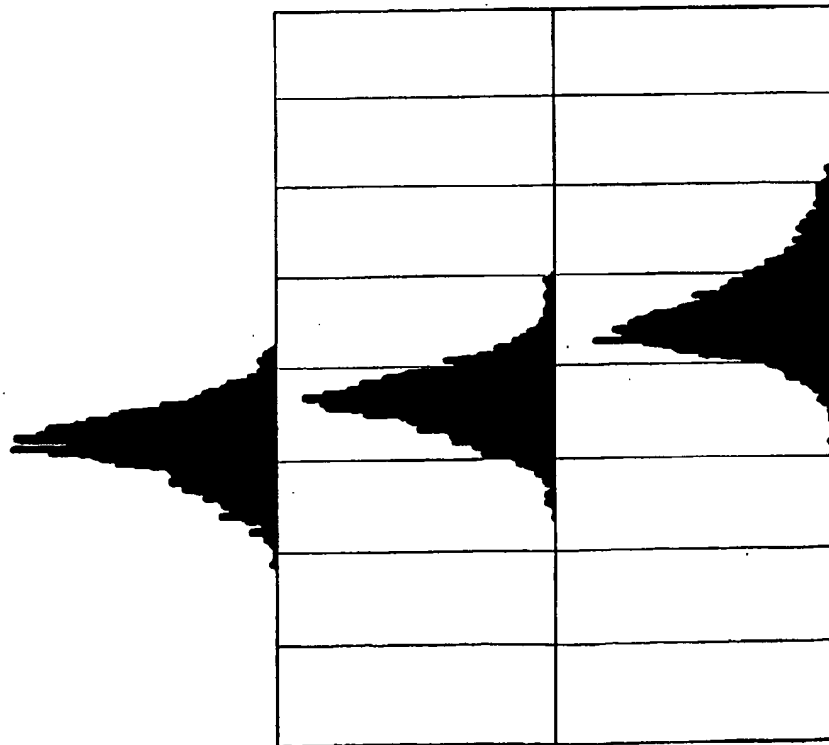
A8-B5 (mean = 45.0)
Average

B7-D10 (mean = 73.18)
Medium

B7-E6 (mean = 233.0)
Super Clone!

• Subclones of transfected clones obtained by
FACS sorting into 96 well plates

FIG.5

Human EphA2 Protein Expression in
CT26 Murine Colon Carcinoma Cells
Following FACS Sorting

pooled populations of transfected
cells sorted by FACS
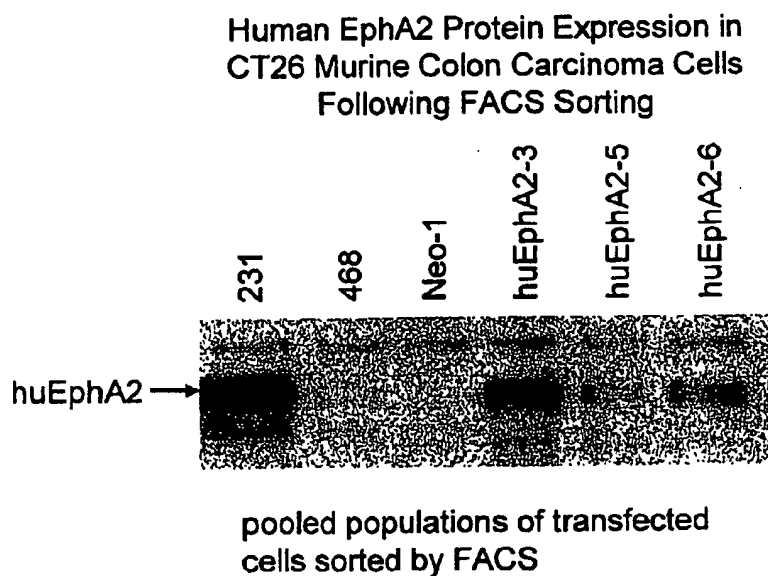
FIG.6

Human EphA2 Protein Expression in B16F10 Cells
Round 3

Anti-EphA2 + 2°(mean = 42.4)

2°-control (mean = 5.58)

Unstained (mean = 4.73)

FIG.7

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Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

FIG.8

FIG.9A

FIG.9B

FIG.10A

* p=0.0012

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FIG.10B

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 huEphA2 Tumor Growth
Prevention Study
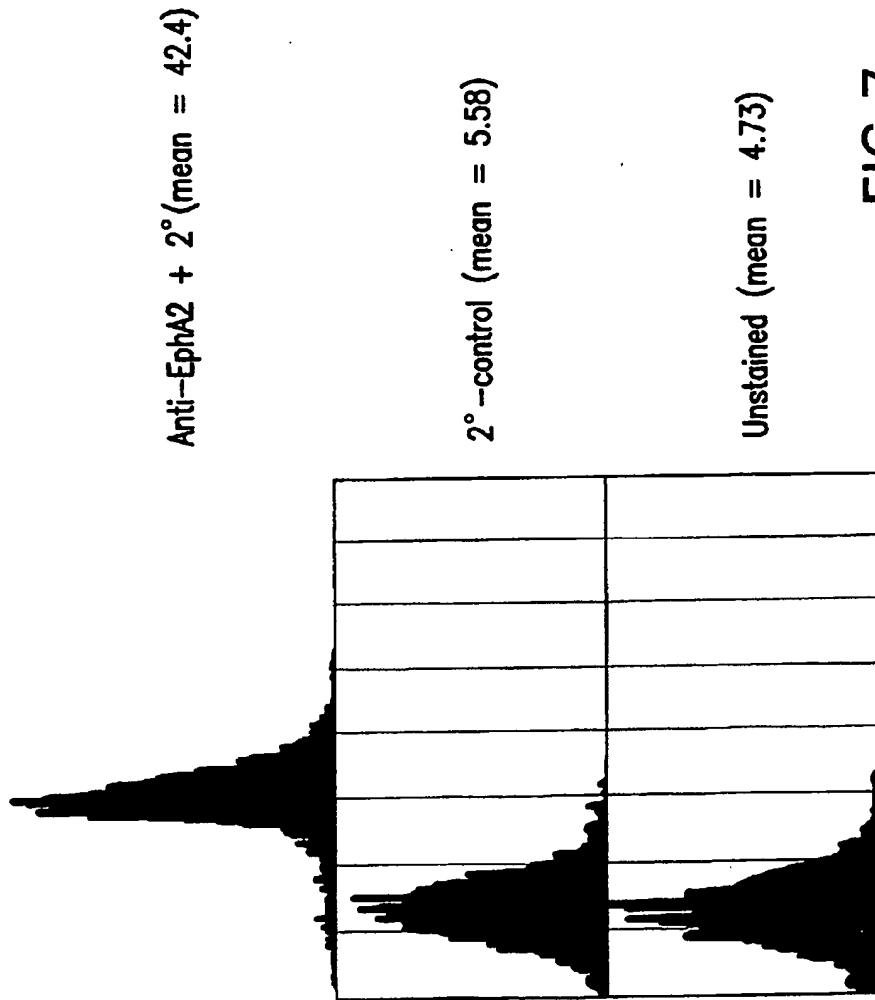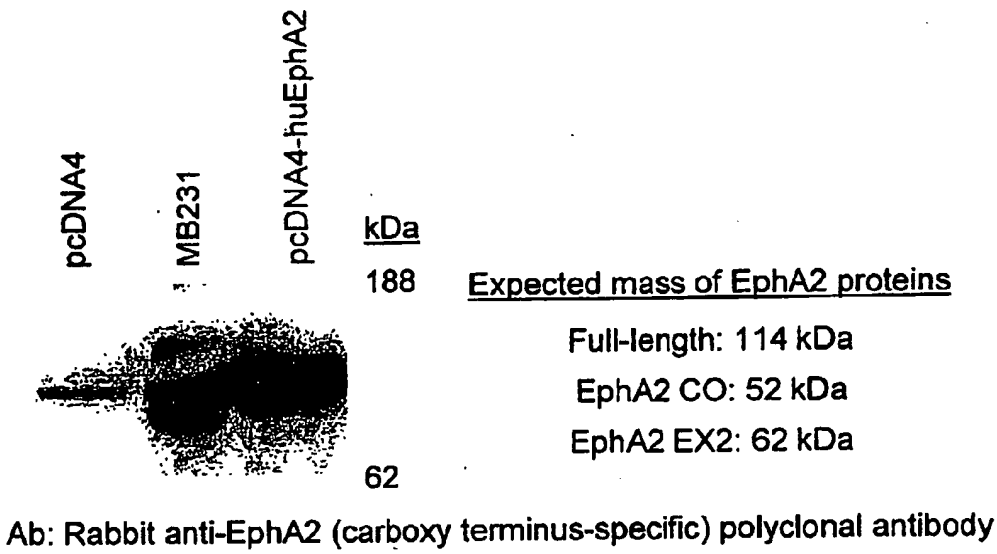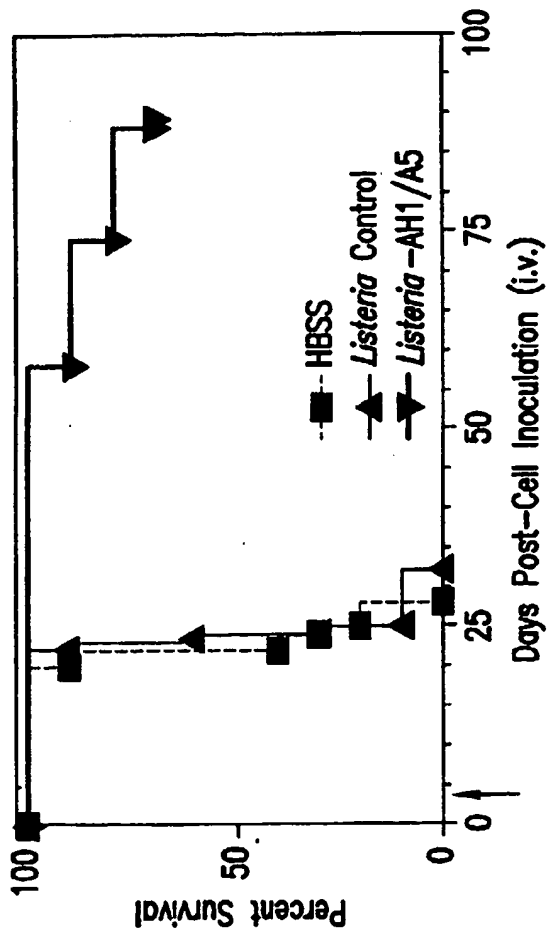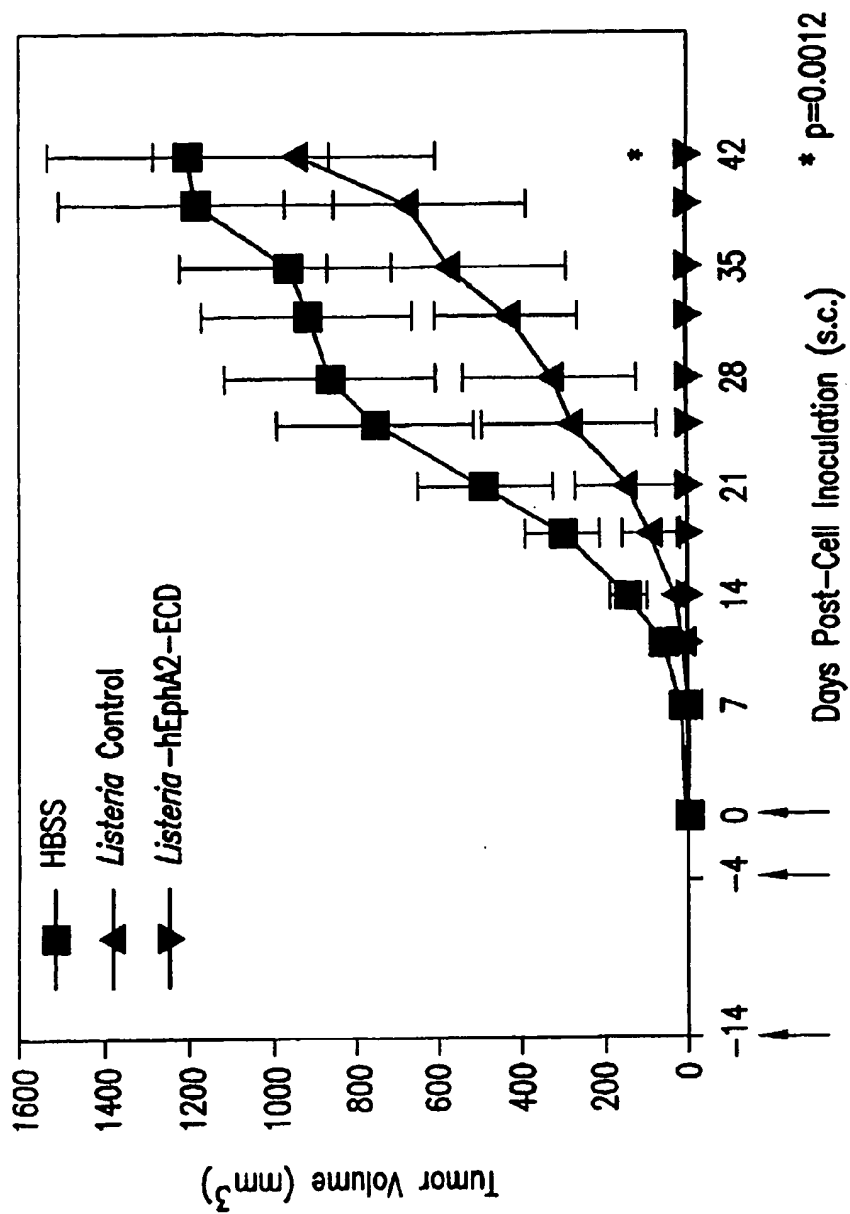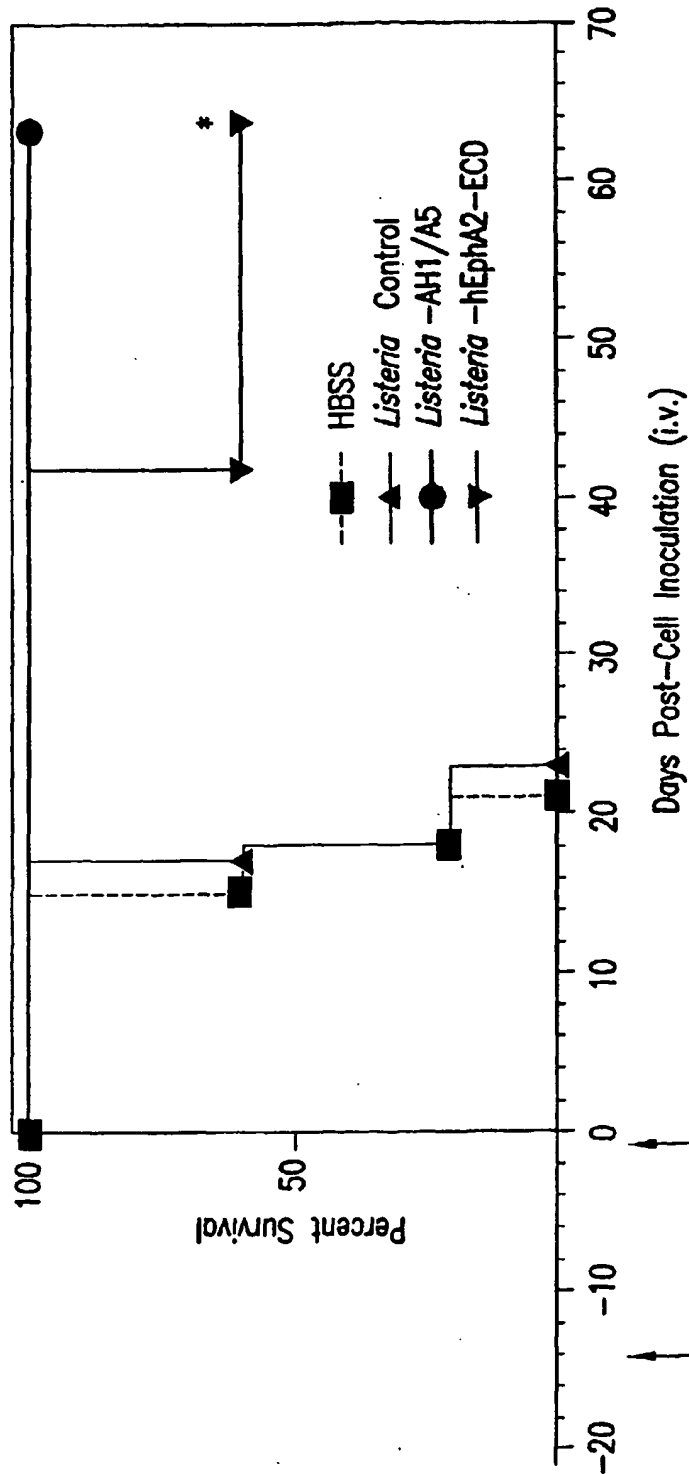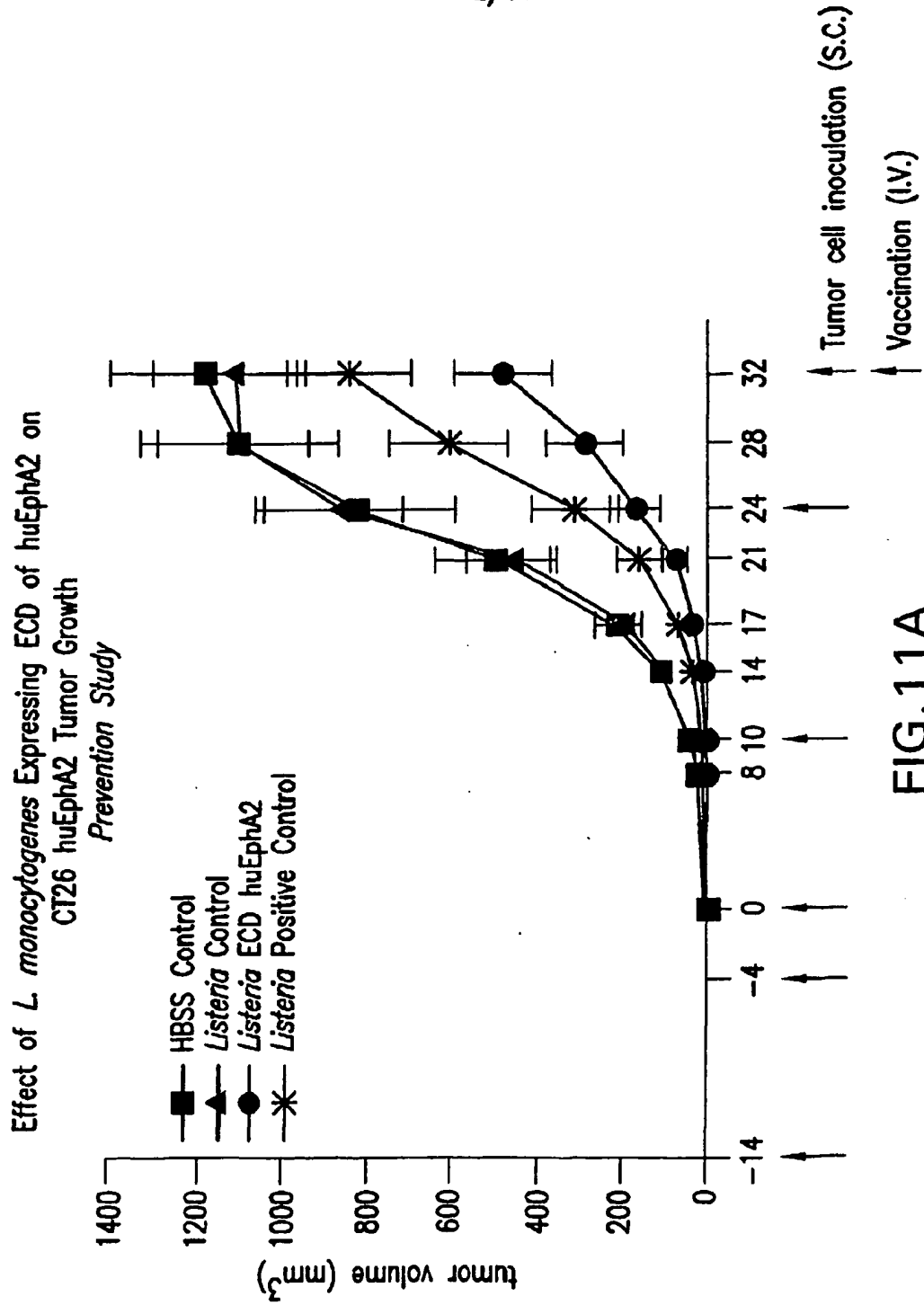
FIG.11A

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 huEphA2 Tumor Growth
Prevention Study

FIG.11B

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FIG.11C

Animal Survival in CT26 huEphA2
Lung Metastases Model
Prevention Study

FIG.11D

HBSS Control
Listeria Control
Listeria-ECD huEphA2
Positive Control *Listeria*

Tumor cell inoculation (S.C.)
Vaccination (I.V.)

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FIG.12

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 Tumor Growth
Therapeutic Study

FIG.13A

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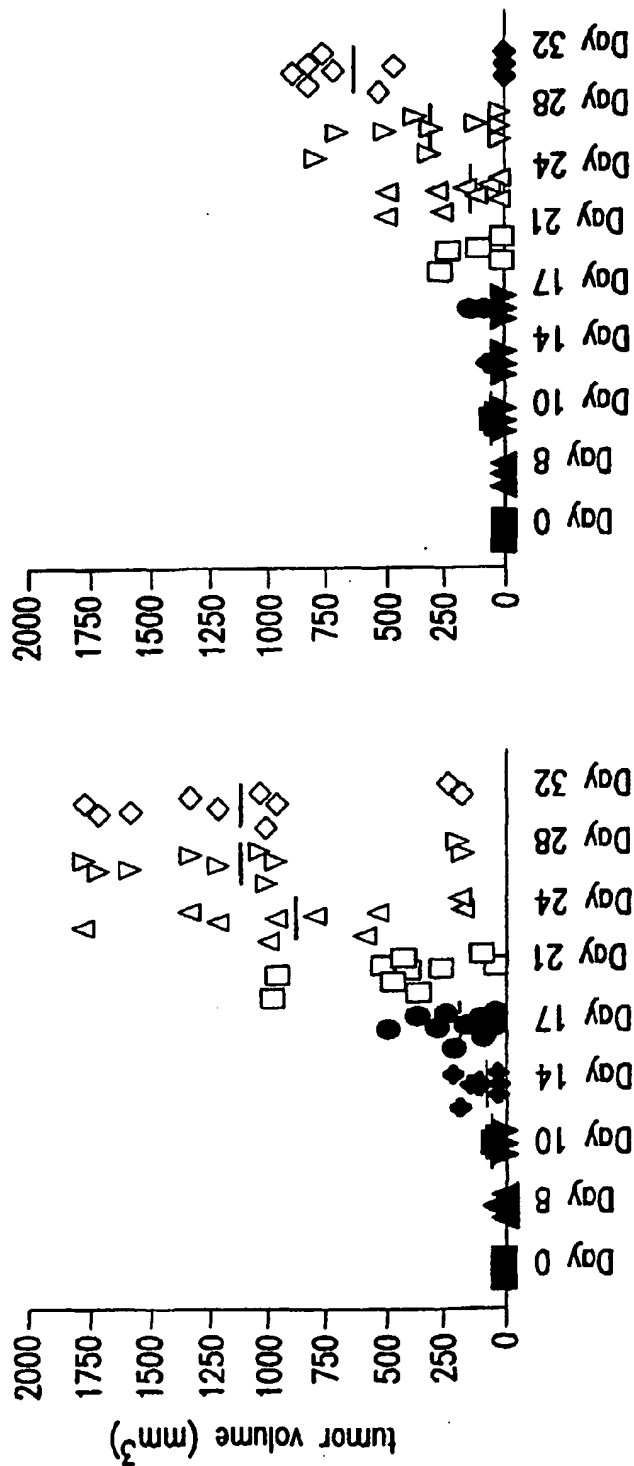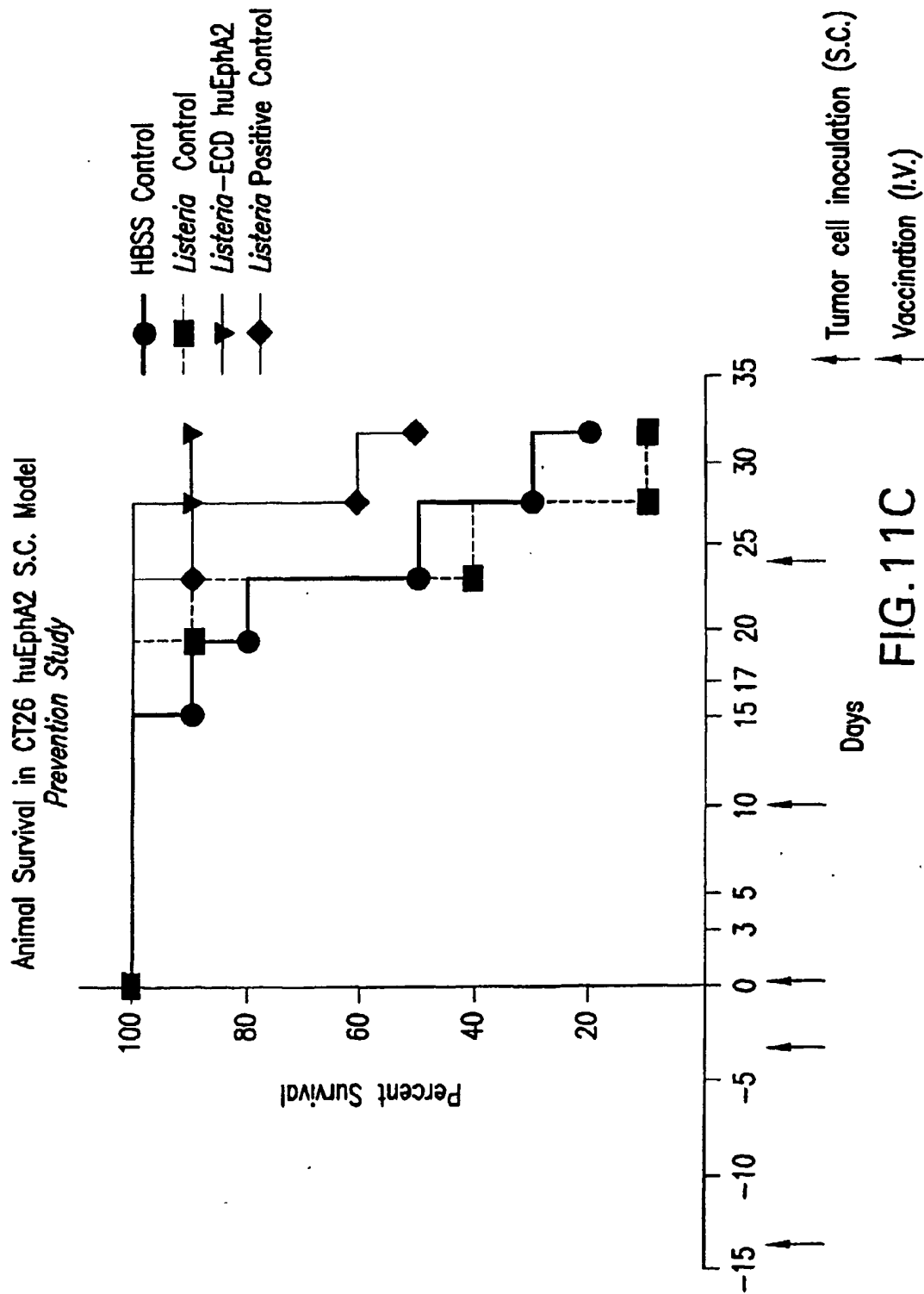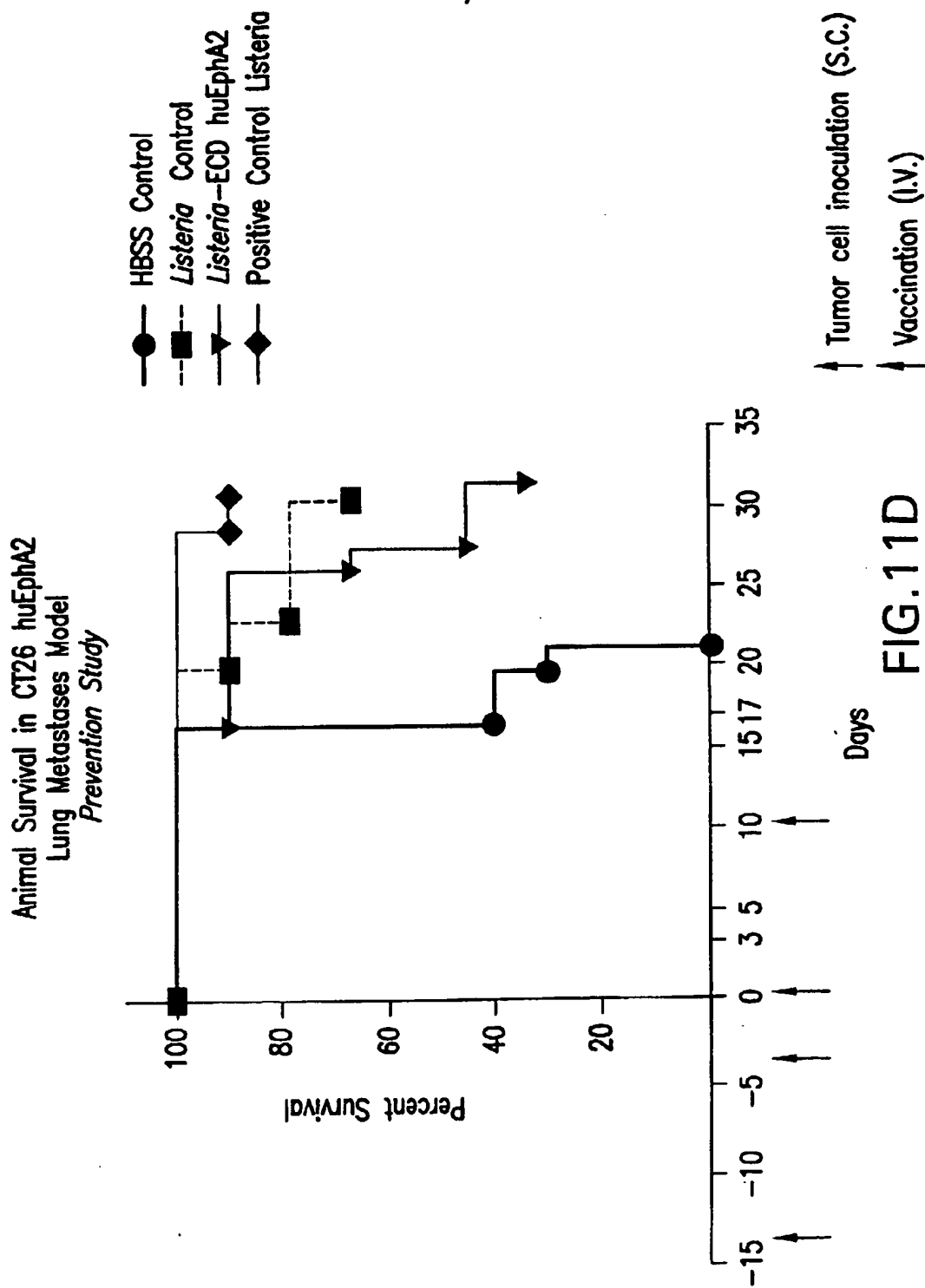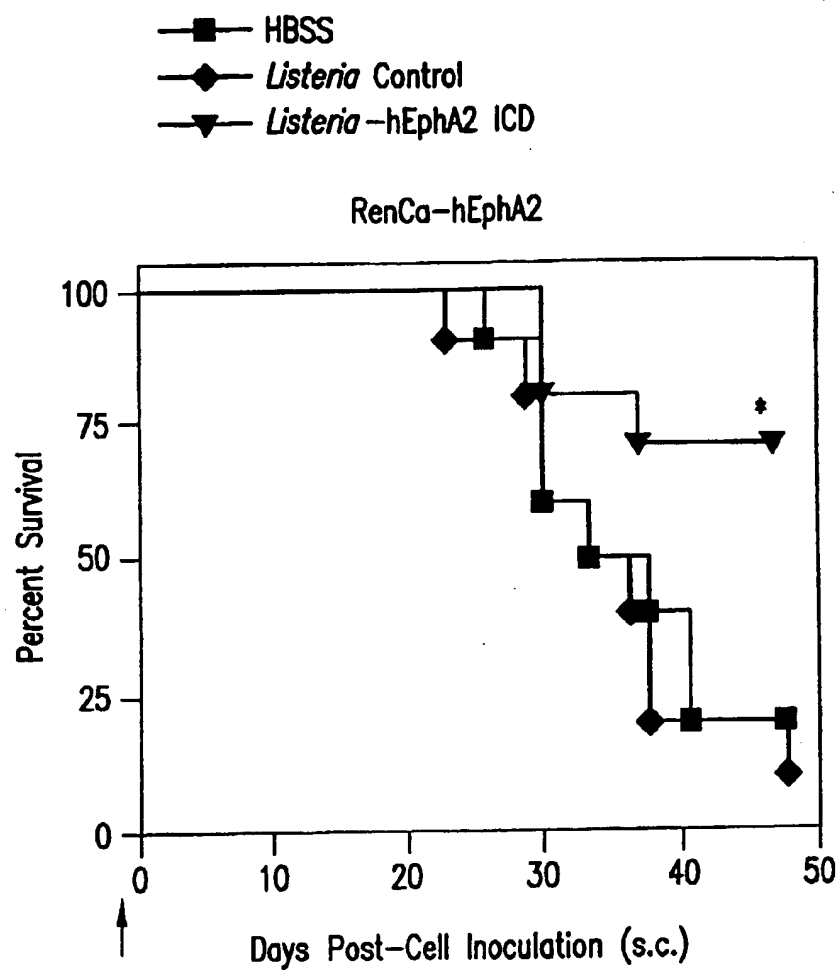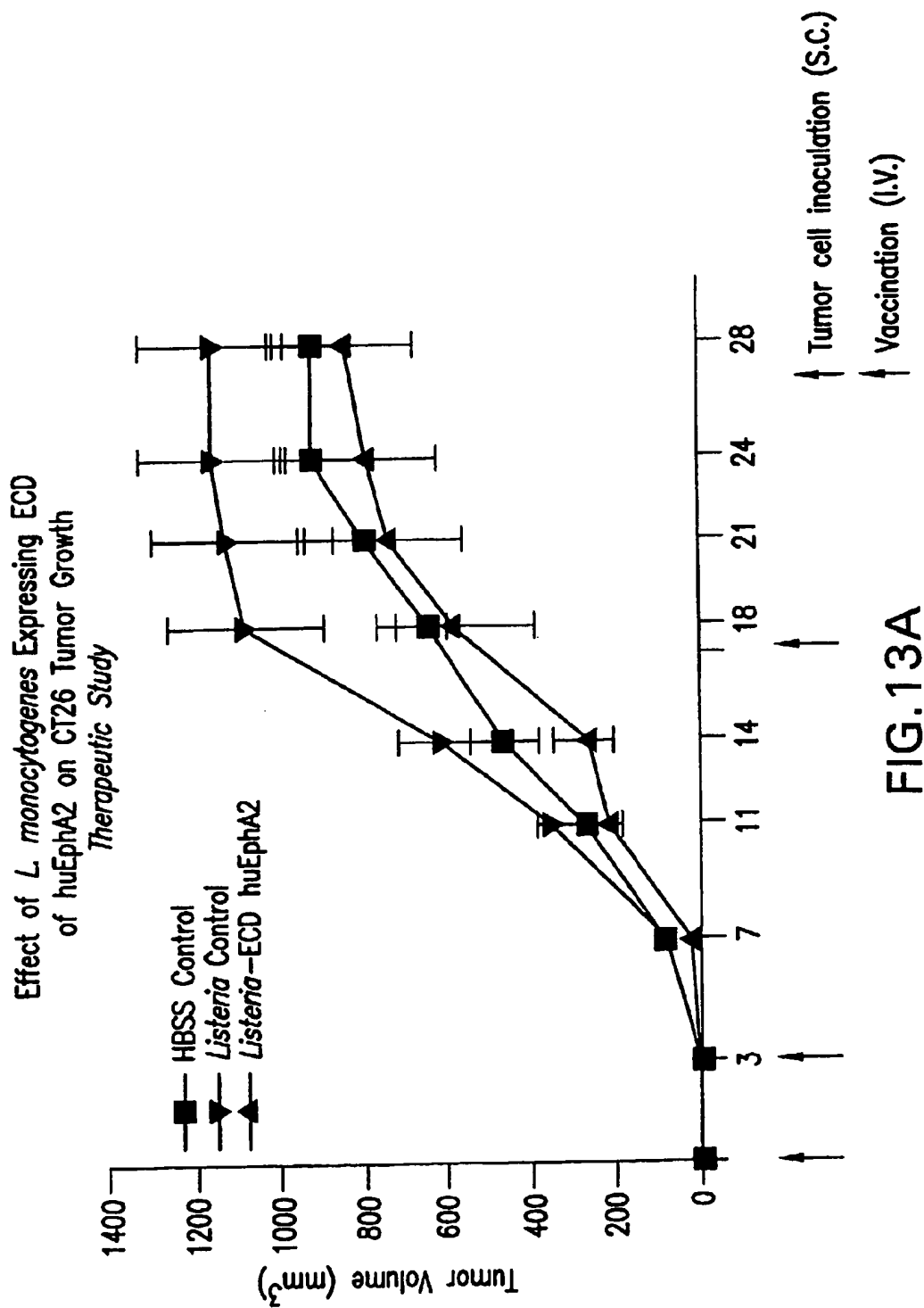
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Therapeutic Study
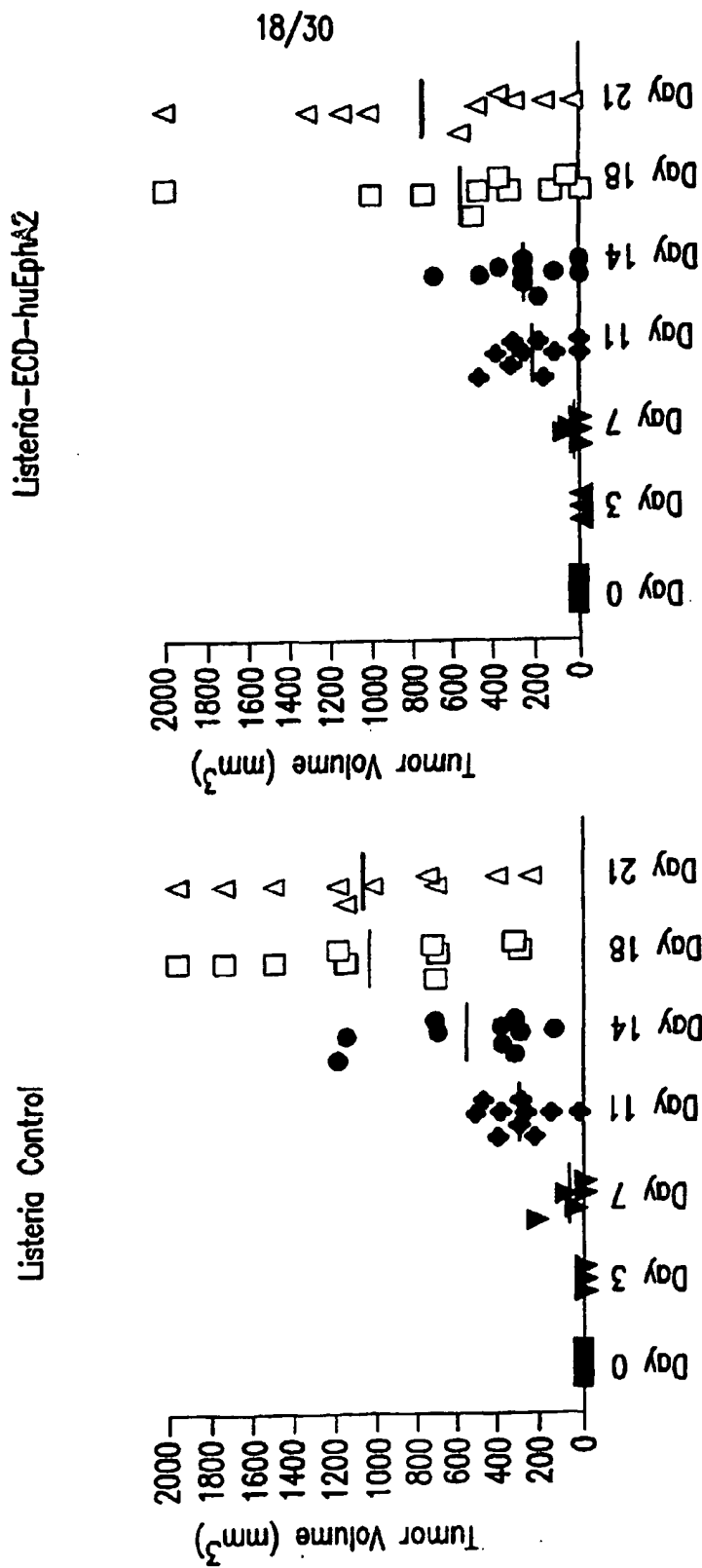
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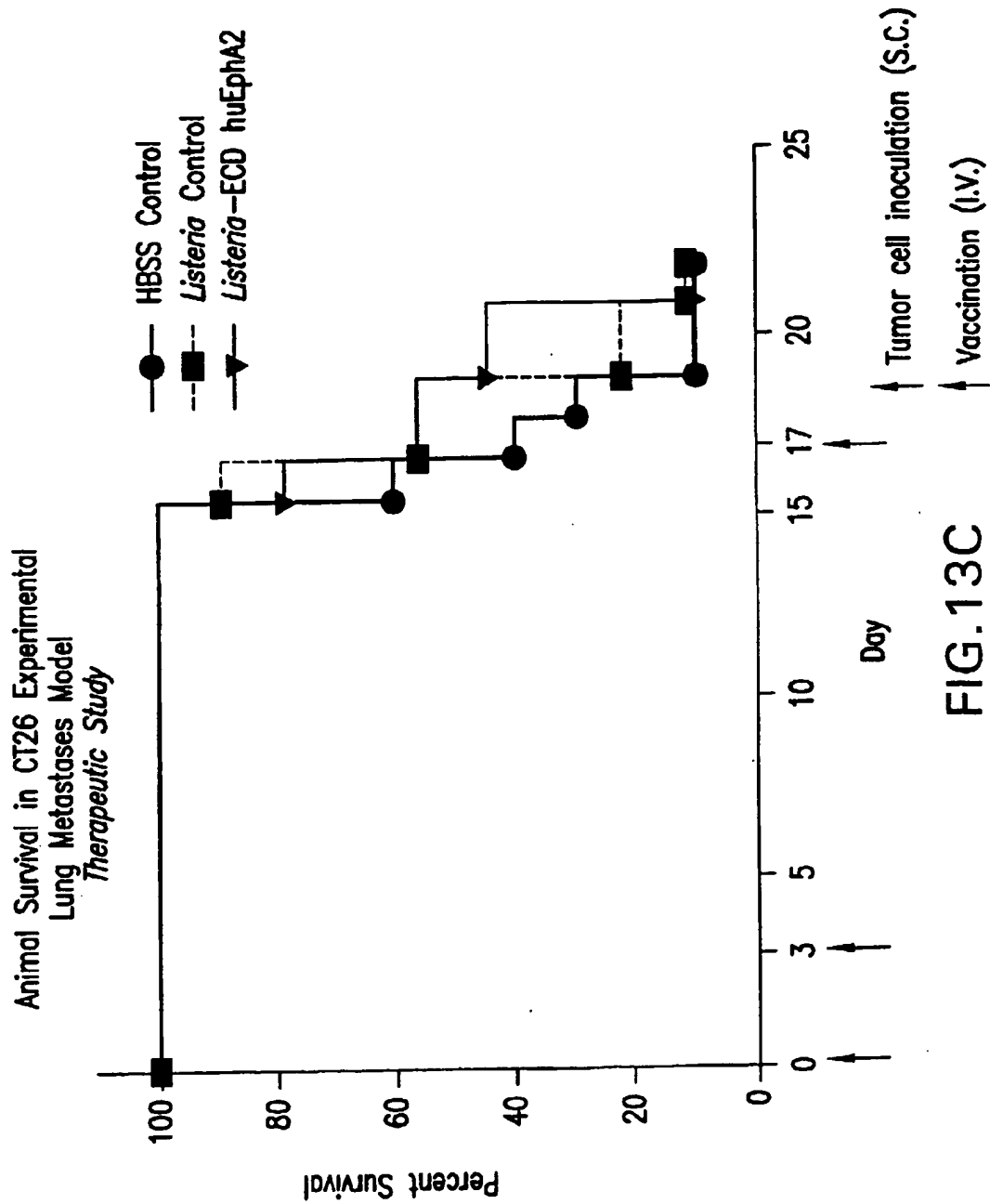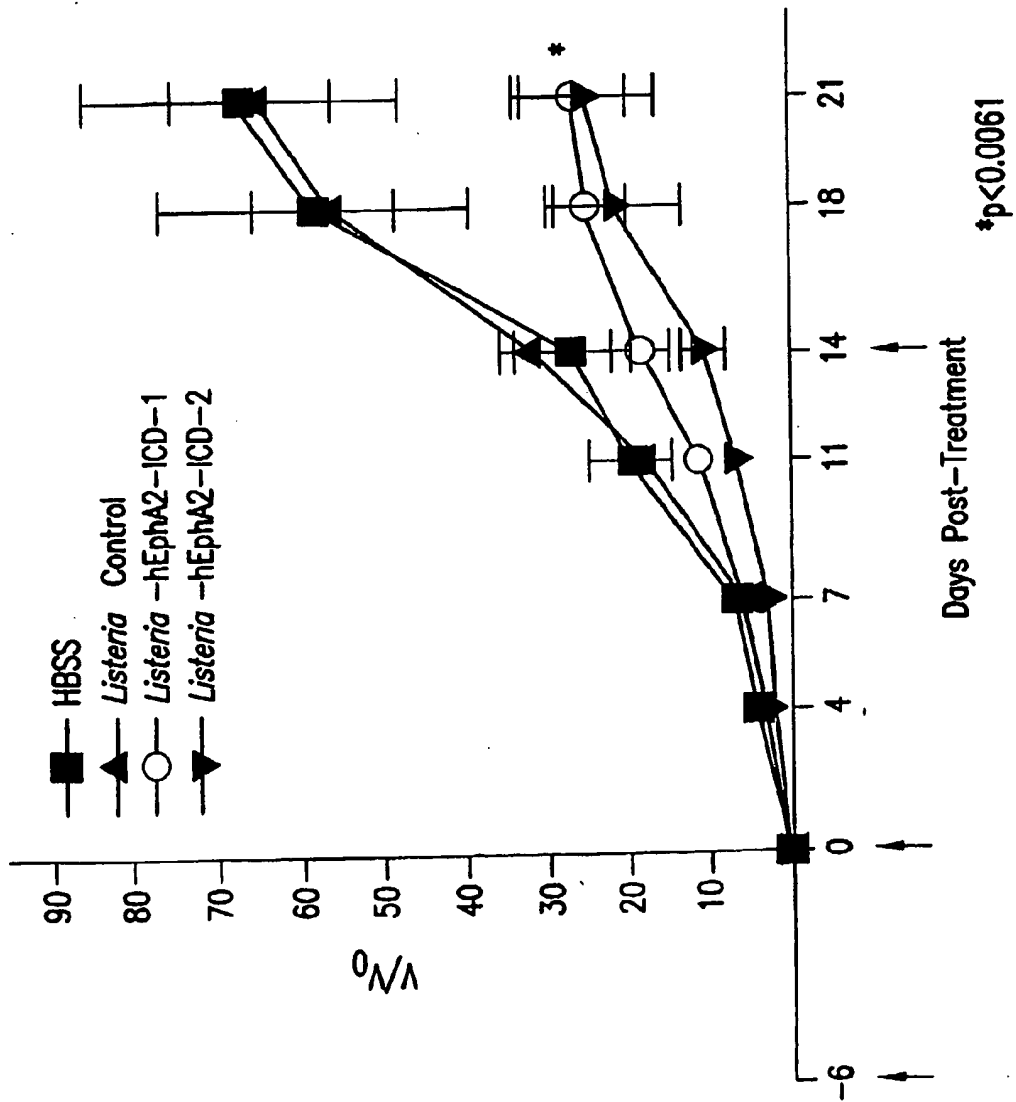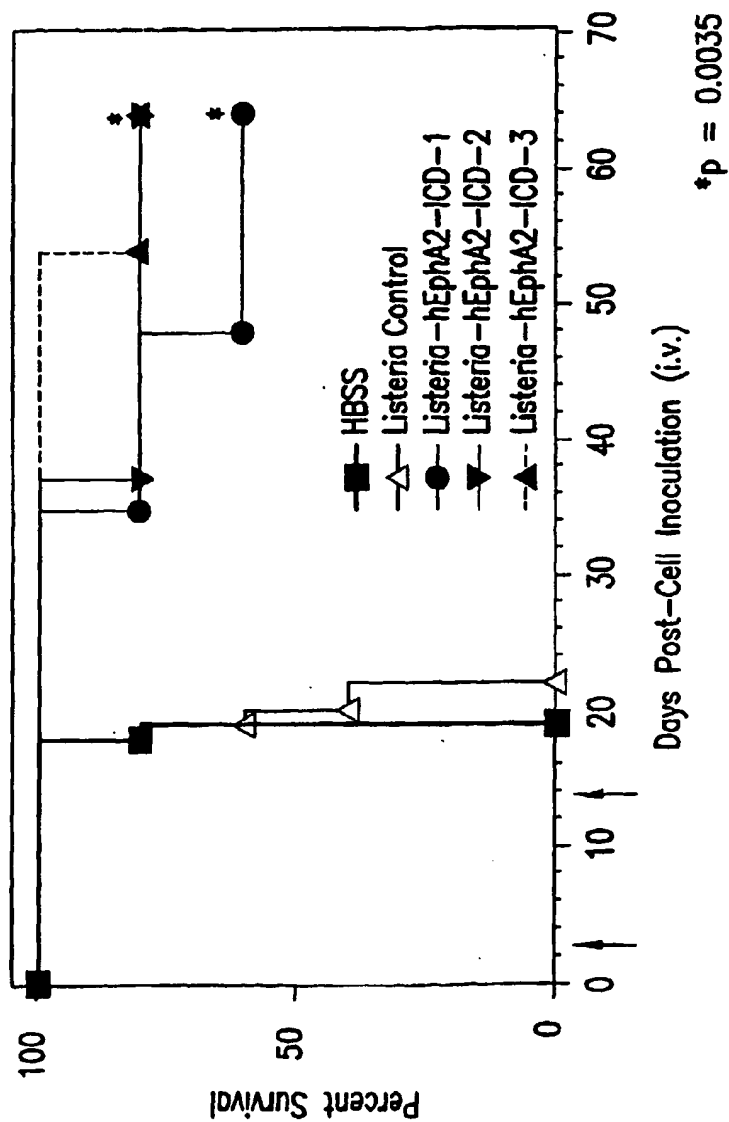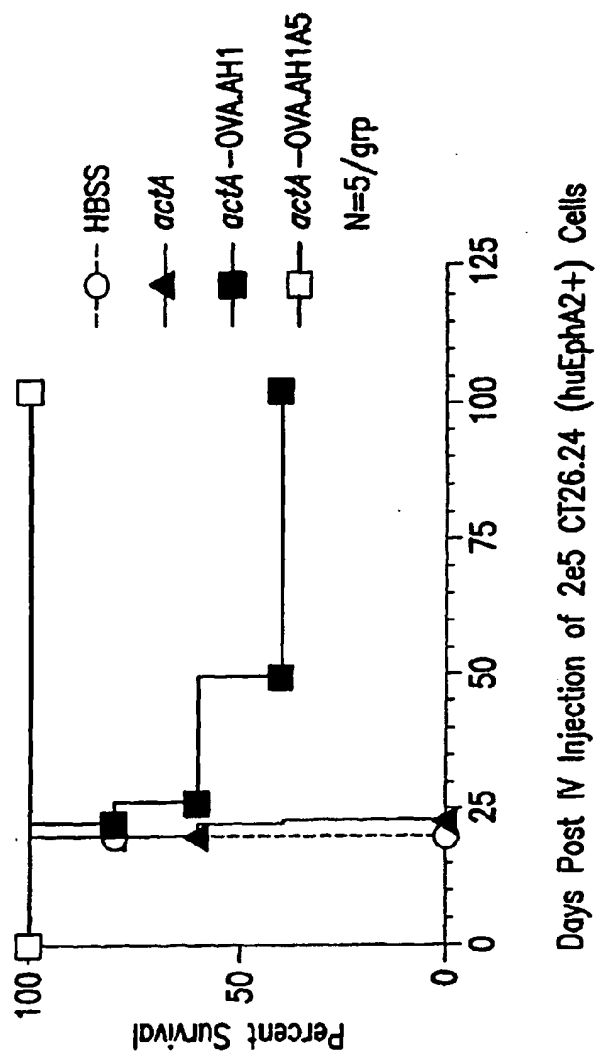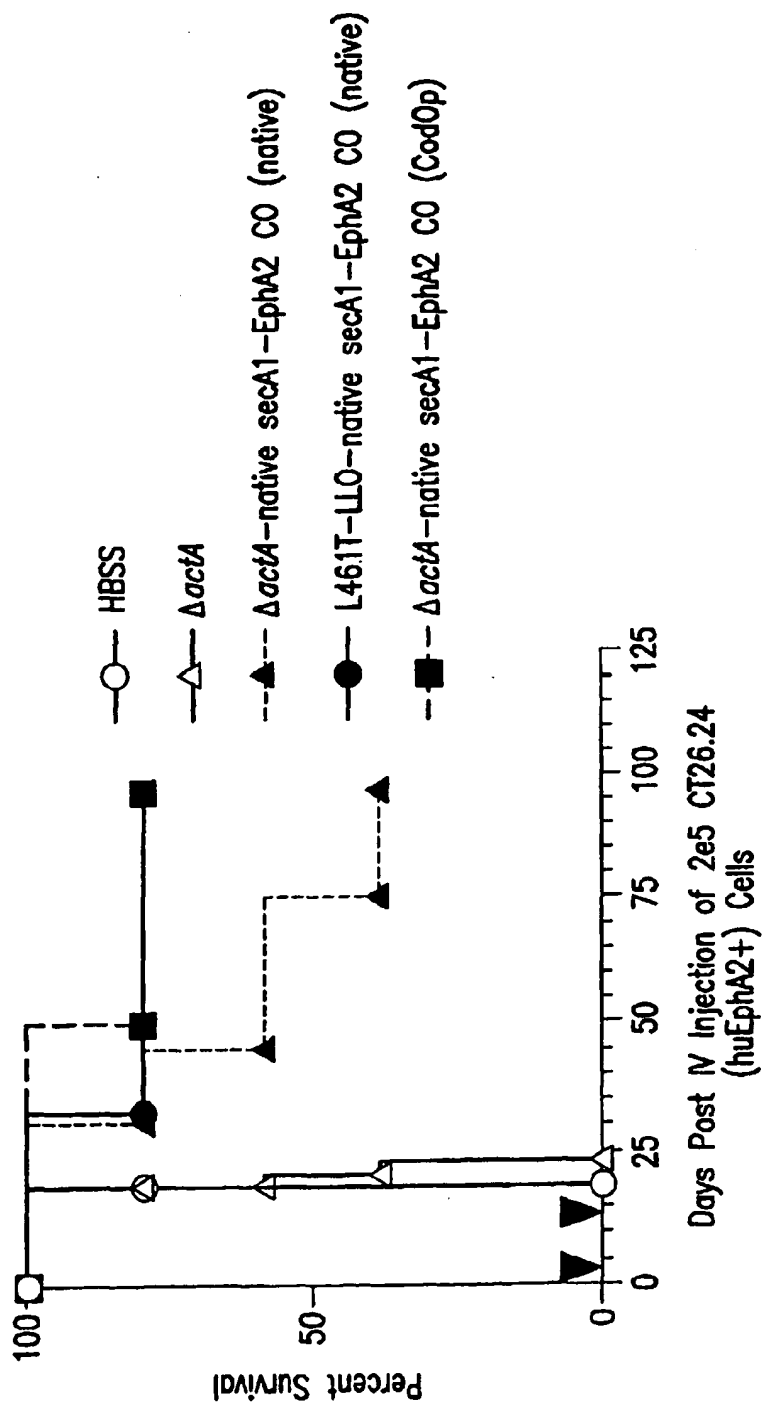
Animal Survival in CT26 Experimental
Lung Metastases Model
Therapeutic Study

FIG.13C

FIG.14A

FIG.14B

FIG.14C

FIG.14D

FIG.14E

FIG.14F

FIG.15

FIG.16

FIG.17

Listeria Control   Listeria-hEphA2 ECD

Listeria-hEphA2 ICD
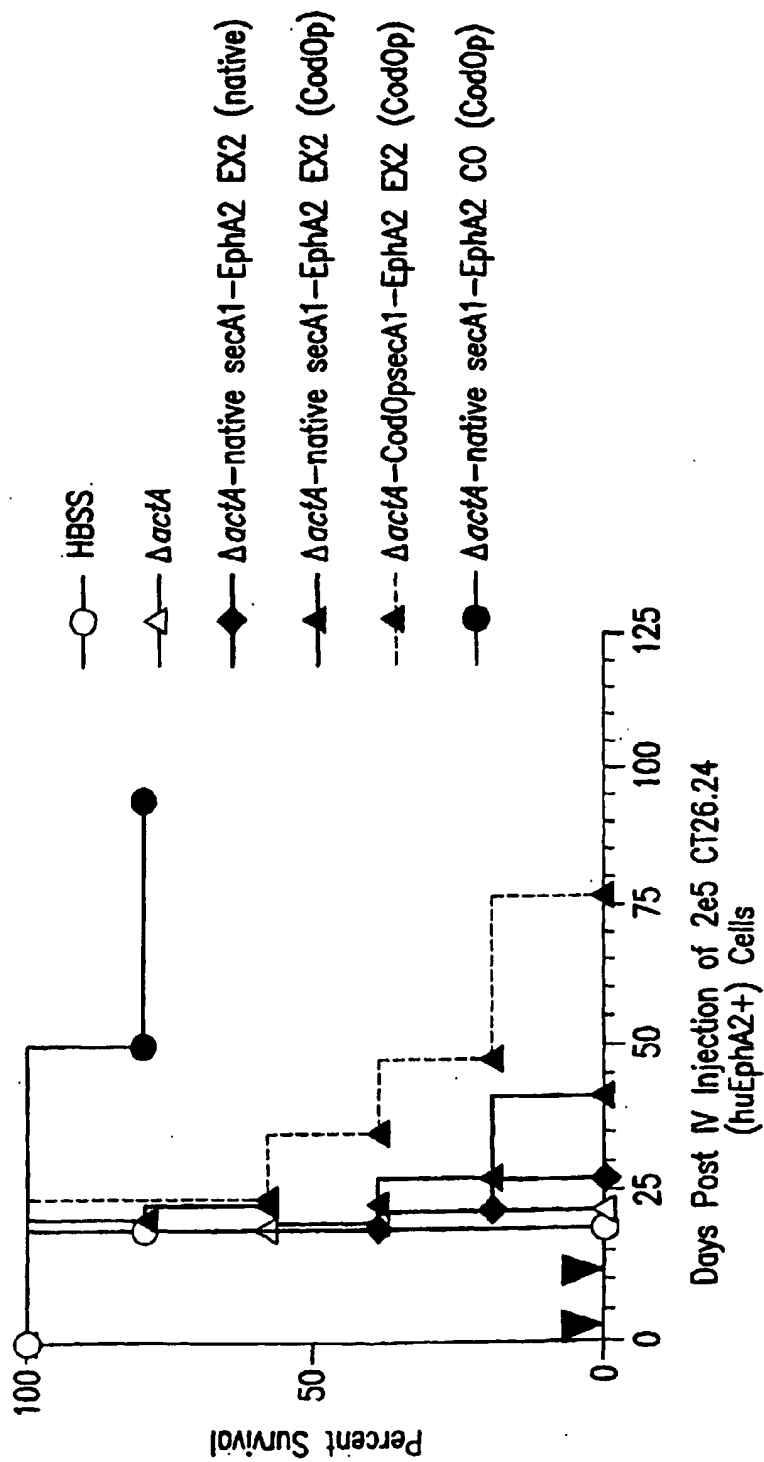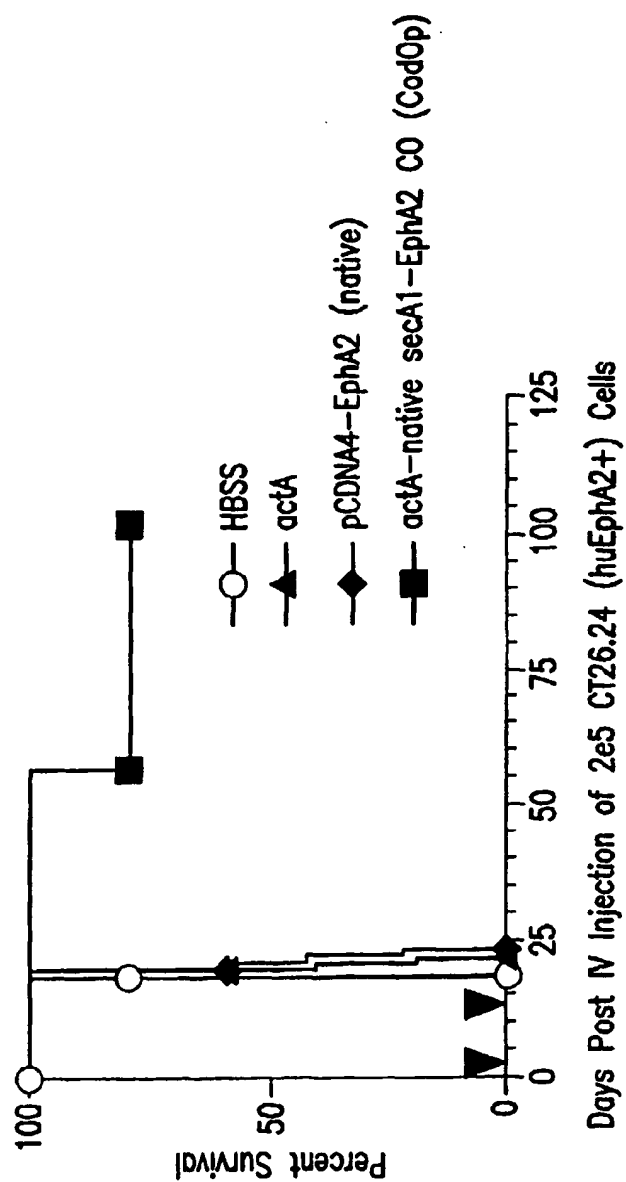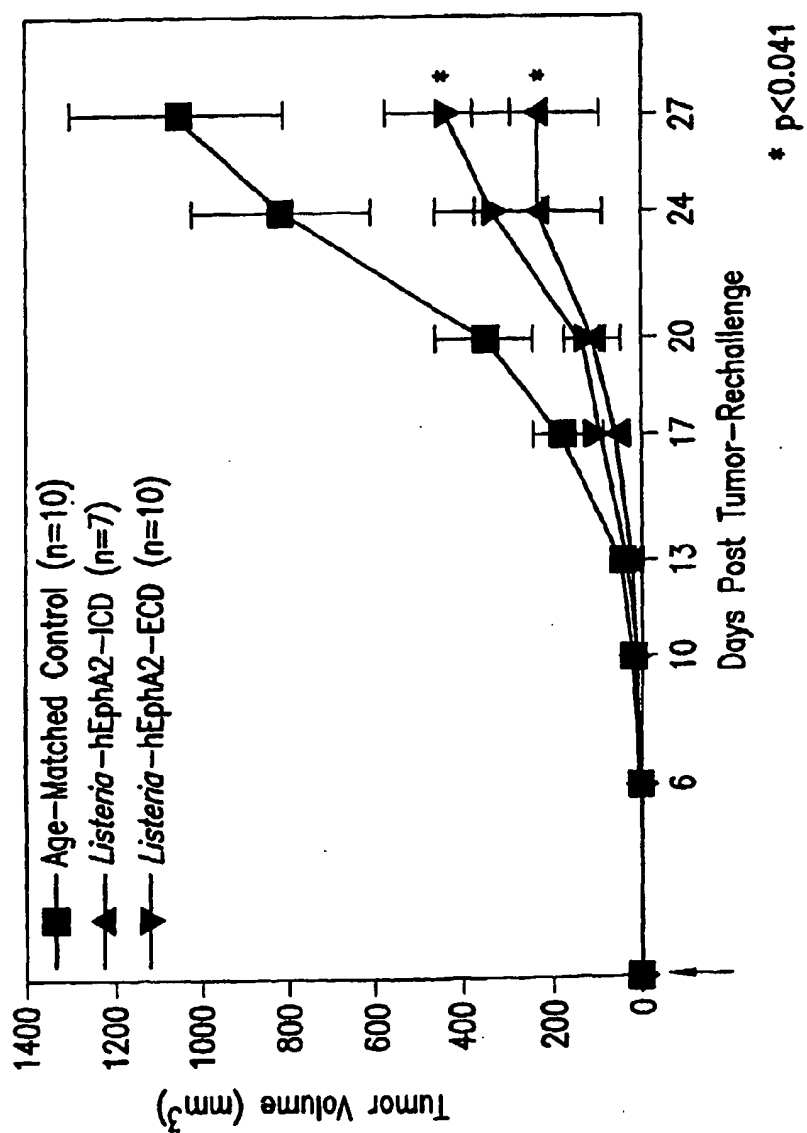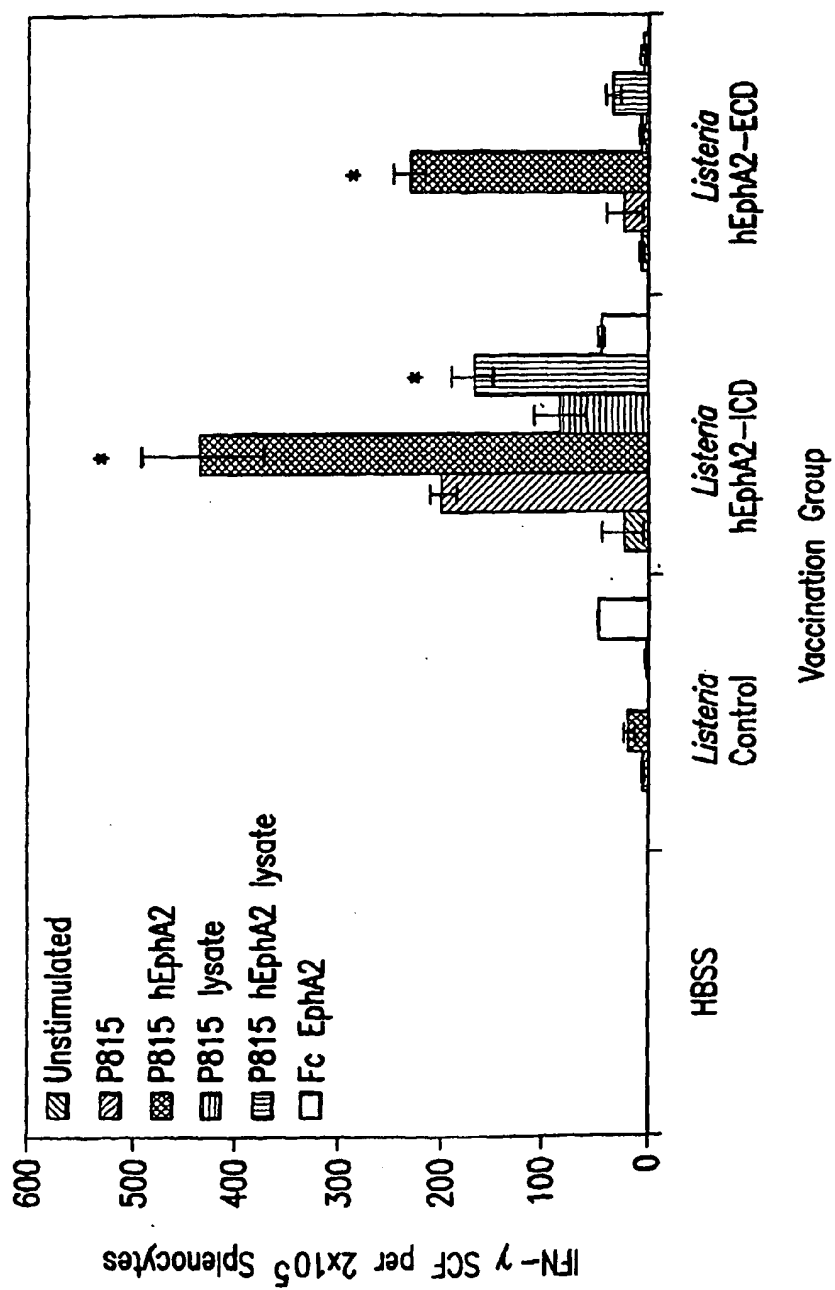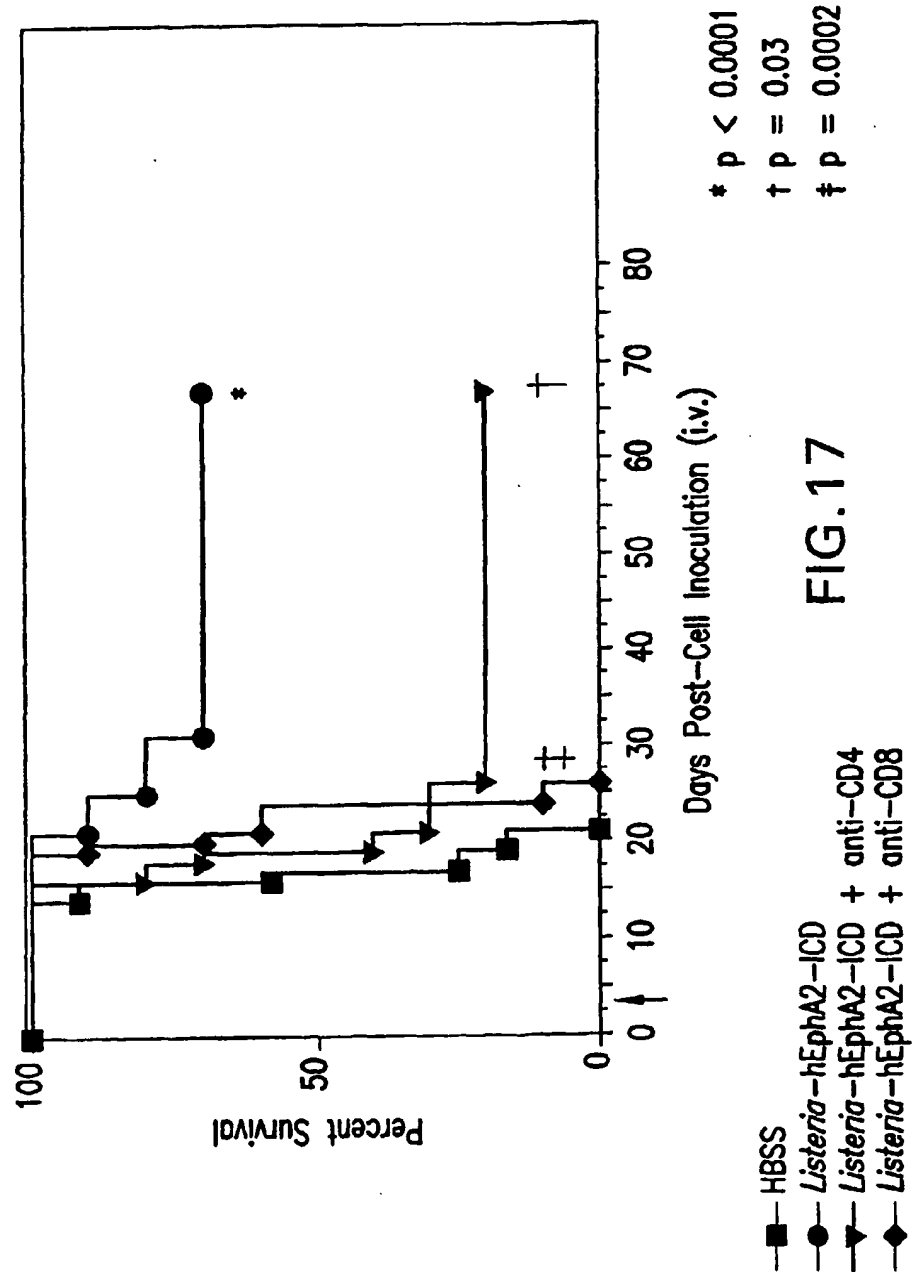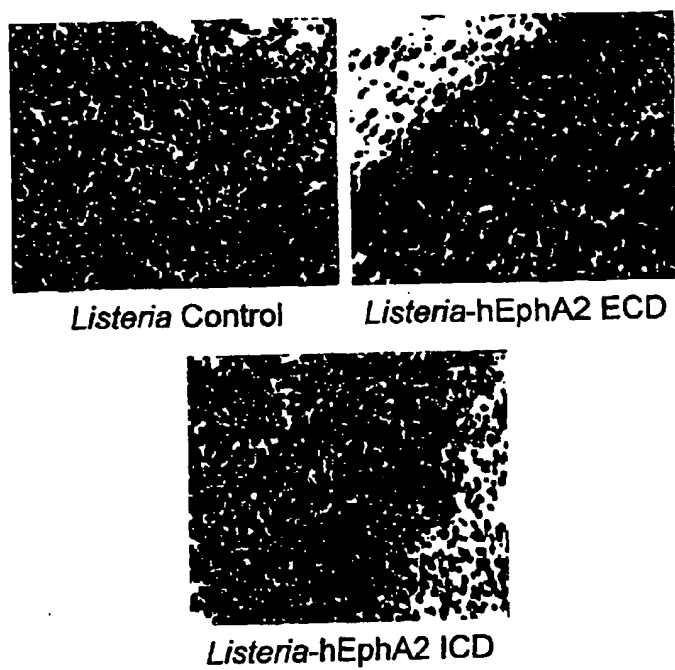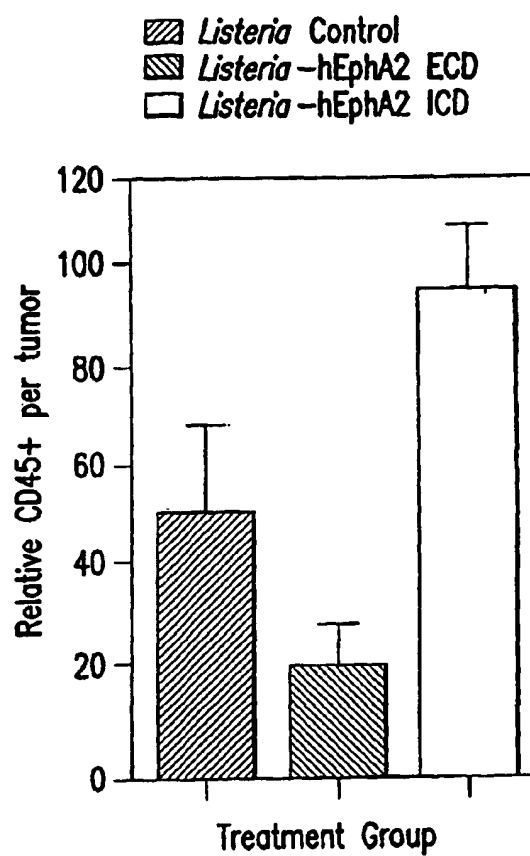
FIG.18A

FIG.18B

SEQUENCE LISTING

<110> Kinch, Michael S.

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<150> US 60/532,696
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                   Met Glu Leu Gln Ala Ala Arg Ala Cys Phe Ala
                    1   5   10

ctg ctg tgg ggc tgt gcg ctg gcc gcg gcc gcg gcg gcg cag ggc aag   218
Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala Ala Ala Ala Gln Gly Lys
            15   20   25

gaa gtg gta ctg ctg gac ttt gct gca gct gga ggg gag ctc ggc tgg   266
Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp
        30   35   40

ctc aca cac ccg tat ggc aaa ggg tgg gac ctg atg cag aac atc atg   314
Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met
    45   50   55

aat gac atg ccg atc tac atg tac tcc gtg tgc aac gtg atg tct ggc   362
Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
60   65   70   75

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Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala

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Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser
            95                  100                 105

ttc cct ggt ggc gcc agc tcc tgc aag gag act ttc aac ctc tac tat      506
Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr
            110                 115                 120

gcc gag tcg gac ctg gac tac ggc acc aac ttc cag aag cgc ctg ttc      554
Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe
            125                 130                 135

acc aag att gac acc att gcg ccc gat gag atc acc gtc agc agc gac      602
Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp
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Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly
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ccg ctc acc cgc aaa ggc ttc tac ctg gcc ttc cag gat atc ggt gcc      698
Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala
            175                 180                 185

tgt gtg gcg ctg ctc tcc gtc cgt gtc tac tac aag aag tgc ccc gag      746
Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu
            190                 195                 200

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Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp
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Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala Val
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gtg cca ccg ggg ggt gaa gag ccc cgt atg cac tgt gca gtg gat ggc      890
Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
            240                 245                 250

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Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
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Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
            270                 275                 280

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Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro
            285                 290                 295

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Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
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Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
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Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
        365             370             375

gag gcc agt gtg cgc tac tcg gag cct cct cac gga ctg acc cgc acc        1322
Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
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Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
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Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
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Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
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Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
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Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu
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Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly
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Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala Gly Val Gly
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Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys
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780                 785                 790                 795

tct gcc agc gac gtg tgg agc ttt ggc att gtc atg tgg gag gtg atg      2570
Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu Val Met
                800                 805                 810

acc tat ggc gag cgg ccc tac tgg gag ttg tcc aac cac gag gtg atg      2618
```

```
Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met
        815             820             825
```

```
aaa gcc atc aat gat ggc ttc cgg ctc ccc aca ccc atg gac tgc ccc    2666
Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro
        830             835             840
```

```
tcc gcc atc tac cag ctc atg atg cag tgc tgg cag cag gag cgt gcc    2714
Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala
        845             850             855
```

```
cgc cgc ccc aag ttc gct gac atc gtc agc atc ctg gac aag ctc att    2762
Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile
860             865             870             875
```

```
cgt gcc cct gac tcc ctc aag acc ctg gct gac ttt gac ccc cgc gtg    2810
Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val
            880             885             890
```

```
tct atc cgg ctc ccc agc acg agc ggc tcg gag ggg gtg ccc ttc cgc    2858
Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg
            895             900             905
```

```
acg gtg tcc gag tgg ctg gag tcc atc aag atg cag cag tat acg gag    2906
Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu
            910             915             920
```

```
cac ttc atg gcg gcc ggc tac act gcc atc gag aag gtg gtg cag atg    2954
His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met
        925             930             935
```

```
acc aac gac gac atc aag agg att ggg gtg cgg ctg ccc ggc cac cag    3002
Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln
940             945             950             955
```

```
aag cgc atc gcc tac agc ctg ctg gga ctc aag gac cag gtg aac act    3050
Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr
            960             965             970
```

```
gtg ggg atc ccc atc tga gcctcgacag ggcctggagc cccatcggcc          3098
Val Gly Ile Pro Ile
            975
```

```
aagaatactt gaagaaacag agtggcctcc ctgctgtgcc atgctgggcc actggggact    3158

ttatttattt ctagttcttt cctcccctg caacttccgc tgaggggtct cggatgacac     3218

cctggcctga actgaggaga tgaccaggga tgctgggctg ggccctcttt ccctgcgaga    3278

cgcacacagc tgagcactta gcaggcaccg ccacgtccca gcatccctgg agcaggagcc    3338

ccgccacagc cttcggacag acatatagga tattcccaag ccgaccttcc ctccgccttc    3398

tcccacatga ggccatctca ggagatggag ggcttggccc agcgccaagt aaacagggta    3458

cctcaagccc catttcctca cactaagagg gcagactgtg aacttgactg ggtgagaccc    3518

aaagcggtcc ctgtccctct agtgccttct ttagaccctc gggccccatc ctcatccctg    3578

actggccaaa cccttgcttt cctgggcctt tgcaagatgc ttggttgtgt tgaggttttt    3638
```

```
aaatatatat tttgtacttt gtggagagaa tgtgtgtgtg tggcaggggg ccccgccagg    3698

gctggggaca gagggtgtca aacattcgtg agctggggac tcagggaccg gtgctgcagg    3758

agtgtcctgc ccatgcccca gtcggcccca tctctcatcc ttttggataa gtttctattc    3818

tgtcagtgtt aaagattttg ttttgttgga cattttttc gaatcttaat ttattatttt      3878

ttttatattt attgttagaa aatgacttat ttctgctctg gaataaagtt gcagatgatt    3938

caaaccgaaa aaaaaaaaa aaaaa                                            3963
```

<210> 2
<211> 976
<212> PRT
<213> Homo sapiens

<400> 2

```
Met Glu Leu Gln Ala Ala Arg Ala Cys Phe Ala Leu Leu Trp Gly Cys
1               5                   10                  15


Ala Leu Ala Ala Ala Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu
            20                  25                  30


Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr
        35                  40                  45


Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile
    50                  55                  60


Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp
65                  70                  75                  80


Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile
                85                  90                  95


Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala
            100                 105                 110


Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
        115                 120                 125


Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr
    130                 135                 140


Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His
145                 150                 155                 160


Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys
```

```
                165                    170                    175


    Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu
            180                     185                 190


    Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu
            195                 200                     205


    Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala
        210                     215                 220


    Thr Val Ala Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly
    225                     230                 235                 240


    Glu Glu Pro Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro
                    245                 250                 255


    Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala
                260                 265                 270


    Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser
            275                 280                     285


    Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala
        290                     295                 300


    Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro
    305                     310                 315                 320


    Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr
                    325                 330                 335


    Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln
                340                 345                 350


    Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln
            355                 360                 365


    Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg
        370                 375                 380


    Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser
    385                 390                 395                 400


    Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn
                405                 410                 415
```

Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val
            420   425   430

Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser
            435   440   445

Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser
     450   455   460

Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn
465   470   475   480

Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp
                485   490   495

Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln
            500   505   510

Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu Phe Gln Thr Leu Ser
            515   520   525

Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly
     530   535   540

Val Val Leu Leu Leu Val Leu Ala Gly Val Gly Phe Phe Ile His Arg
545   550   555   560

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
            565   570   575

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
            580   585   590

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
            595   600   605

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
     610   615   620

Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
625   630   635   640

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
            645   650   655

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
        660   665   670

His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
        675   680   685

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
        690   695   700

Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
705   710   715   720

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
                725   730   735

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
            740   745   750

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
        755   760   765

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
    770   775   780

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
785   790   795   800

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
            805   810   815

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
        820   825   830

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
        835   840   845

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
    850   855   860

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
865   870   875   880

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
            885   890   895

```
Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
        900             905             910
```

```
Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
        915             920             925
```

```
Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
        930             935             940
```

```
Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
945             950             955             960
```

```
Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
            965             970             975
```

```
<210>   3
<211>   12
<212>   PRT
<213>   Homo sapiens

<400>   3

Thr Leu Ala Asp Phe Asp Pro Arg Val Pro Arg Thr
1               5               10
```

```
<210>   4
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   4

Val Leu Leu Leu Val Leu Ala Gly Val
1               5
```

```
<210>   5
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   5

Val Leu Ala Gly Val Gly Phe Phe Ile
1               5
```

```
<210>   6
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   6
```

Ile Met Asn Asp Met Pro Ile Tyr Met
1   5

<210> 7
<211> 9
<212> PRT
<213> Homo sapiens

<400> 7

Ser Leu Leu Gly Leu Lys Asp Gln Val
1   5

<210> 8
<211> 9
<212> PRT
<213> Homo sapiens .

<400> 8

Trp Leu Val Pro Ile Gly Gln Cys Leu
1   5

<210> 9
<211> 9
<212> PRT
<213> Homo sapiens

<400> 9

Leu Leu Trp Gly Cys Ala Leu Ala Ala
1   5

<210> 10
<211> 9
<212> PRT
<213> Homo sapiens

<400> 10

Gly Leu Thr Arg Thr Ser Val Thr Val
1   5

<210> 11
<211> 9
<212> PRT
<213> Homo sapiens

<400> 11

Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
1   5

<210> 12

```
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   12

Lys Leu Asn Val Glu Glu Arg Ser Val
1               5


<210>   13
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   13

Ile Met Gly Gln Phe Ser His His Asn
1               5


<210>   14
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   14

Tyr Ser Val Cys Asn Val Met Ser Gly
1               5


<210>   15
<211>   9
<212>   PRT
<213>   Homo sapiens

<400>   15

Met Gln Asn Ile Met Asn Asp Met Pro
1               5


<210>   16
<211>   15
<212>   PRT
<213>   Homo sapiens

<400>   16

Glu Ala Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg
1               5                   10                  15


<210>   17
<211>   13
<212>   PRT
<213>   Homo sapiens

<400>   17
```

Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
1   5   10

<210> 18
<211> 16
<212> PRT
<213> Homo sapiens

<400> 18

Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser
1   5   10   15

<210> 19
<211> 3105
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct

<400> 19
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa   60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca   120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc   180

gagctccagg cagcccgcgc ctgcttcgcc ctgctgtggg gctgtgcgct ggccgcggcc   240

gcggcggcgc agggcaagga agtggtactg ctggactttg ctgcagctgg aggggagctc   300

ggctggctca cacacccgta tggcaaaggg tgggacctga tgcagaacat catgaatgac   360

atgccgatct acatgtactc cgtgtgcaac gtgatgtctg gcgaccagga caactggctc   420

cgcaccaact gggtgtaccg aggagaggct gagcgtatct tcattgagct caagtttact   480

gtacgtgact gcaacagctt ccctggtggc gccagctcct gcaaggagac tttcaacctc   · 540

tactatgccg agtcggacct ggactacggc accaacttcc agaagcgcct gttcaccaag   600

attgacacca ttgcgcccga tgagatcacc gtcagcagcg acttcgaggc acgccacgtg   660

aagctgaacg tggaggagcg ctccgtgggg ccgctcaccc gcaaaggctt ctacctggcc   720

ttccaggata tcggtgcctg tgtggcgctg ctctccgtcc gtgtctacta caagaagtgc   780

cccgagctgc tgcagggcct ggcccacttc cctgagacca tcgccggctc tgatgcacct   840

tccctggcca ctgtggccgg cacctgtgtg accatgccg tggtgccacc ggggggtgaa   900

gagccccgta tgcactgtgc agtggatggc gagtggctgg tgcccattgg gcagtgcctg   960

tgccaggcag gctacgagaa ggtggaggat gcctgccagg cctgctcgcc tggatttttt   1020

aagtttgagg catctgagag cccctgcttg gagtgccctg agcacacgct gccatcccct   1080

```
gagggtgcca cctcctgcga gtgtgaggaa ggcttcttcc gggcacctca ggacccagcg   1140

tcgatgcctt gcacacgacc cccctccgcc ccacactacc tcacagccgt gggcatgggt   1200

gccaaggtgg agctgcgctg gacgccccct caggacagcg ggggccgcga ggacattgtc   1260

tacagcgtca cctgcgaaca gtgctggccc gagtctgggg aatgcgggcc gtgtgaggcc   1320

agtgtgcgct actcggagcc tcctcacgga ctgacccgca ccagtgtgac agtgagcgac   1380

ctggagcccc acatgaacta caccttcacc gtggaggccc gcaatggcgt ctcaggcctg   1440

gtaaccagcc gcagcttccg tactgccagt gtcagcatca accagacaga gcccccaag    1500

gtgaggctgg agggccgcag caccacctcg cttagcgtct cctggagcat cccccgccg    1560

cagcagagcc gagtgtggaa gtacgaggtc acttaccgca agaagggaga ctccaacagc   1620

tacaatgtgc gccgcaccga gggtttctcc gtgaccctgg acgacctggc cccagacacc   1680

acctacctgg tccaggtgca ggcactgacg caggagggcc aggggccgg cagcagggtg    1740

cacgaattcc agacgctgtc cccggaggga tctggcaact ggcggtgat tggcggcgtg    1800

gctgtcggtg tggtcctgct tctggtgctg gcaggagttg gcttctttat ccaccgcagg   1860

aggaagaacc agcgtgcccg ccagtccccg gaggacgttt acttctccaa gtcagaacaa   1920

ctgaagcccc tgaagacata cgtggacccc cacacatatg aggaccccaa ccaggctgtg   1980

ttgaagttca ctaccgagat ccatccatcc tgtgtcactc ggcagaaggt gatcggagca   2040

ggagagtttg gggaggtgta caagggcatg ctgaagacat cctcggggaa gaaggaggtg   2100

ccggtggcca tcaagacgct gaaagccggc tacacagaga gcagcgagt ggacttcctc    2160

ggcgaggccg gcatcatggg ccagttcagc caccacaaca tcatccgcct agagggcgtc   2220

atctccaaat acaagcccat gatgatcatc actgagtaca tggagaatgg ggccctggac   2280

aagttccttc gggagaagga tggcgagttc agcgtgctgc agctggtggg catgctgcgg   2340

ggcatcgcag ctggcatgaa gtacctggcc aacatgaact atgtgcaccg tgacctggct   2400

gcccgcaaca tcctcgtcaa cagcaacctg gtctgcaagg tgtctgactt tggcctgtcc   2460

cgcgtgctgg aggacgaccc cgaggccacc tacaccacca gtggcggcaa gatccccatc   2520

cgctggaccg ccccggaggc catttcctac cggaagttca cctctgccag cgacgtgtgg   2580

agctttggca ttgtcatgtg ggaggtgatg acctatggcg agcggcccta ctgggagttg   2640

tccaaccacg aggtgatgaa agccatcaat gatggcttcc ggctccccac acccatggac   2700

tgcccctccg ccatctacca gctcatgatg cagtgctggc agcaggagcg tgcccgccgc   2760

cccaagttcg ctgacatcgt cagcatcctg gacaagctca ttcgtgcccc tgactccctc   2820

aagaccctgg ctgactttga ccccgcgtg tctatccggc tccccagcac gagcggctcg    2880

gagggggtgc ccttccgcac ggtgtccgag tggctggagt ccatcaagat gcagcagtat   2940
```

acggagcact tcatggcggc cggctacact gccatcgaga aggtggtgca gatgaccaac   3000

gacgacatca agaggattgg ggtgcggctg cccggccacc agaagcgcat cgcctacagc   3060

ctgctgggac tcaaggacca ggtgaacact gtggggatcc ccatc   3105

<210>   20
<211>   1035
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Description of Artificial Sequence: Predicted fusion protein

<400>   20

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1   5   10   15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20   25   30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
            35   40   45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Leu Gln Ala
        50   55   60

Ala Arg Ala Cys Phe Ala Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala
65   70   75   80

Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala
                85   90   95

Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp
            100   105   110

Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val
            115   120   125

Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp
            130   135   140

Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr
145   150   155   160

Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu
                165   170   175

```
Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn
        180             185             190

Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu
        195             200             205

Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val
    210             215             220

Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala
225             230             235             240

Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr
            245             250             255

Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu
            260             265             270

Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr
        275             280             285

Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met
    290             295             300

His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu
305             310             315             320

Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser
            325             330             335

Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys
        340             345             350

Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys
        355             360             365

Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys
    370             375             380

Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly
385             390             395             400

Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg
            405             410             415
```

Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser
            420   425   430

Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro
            435   440   445

His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His
    450   455   460

Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu
465   470   475   480

Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr
                485   490   495

Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser
            500   505   510

Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr
            515   520   525

Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg
    530   535   540

Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr
545   550   555   560

Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala
                565   570   575

Gly Ser Arg Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly
            580   585   590

Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu
            595   600   605

Val Leu Ala Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln
    610   615   620

Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln
625   630   635   640

Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro
            645   650   655

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Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val
        660   665   670

Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys
        675   680   685

Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile
    690   695   700

Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu
705   710   715   720

Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg
    .   725   730   735

Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu
            740   745   750

Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly
        755   760   765

Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala
    770   775   780

Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala
785   790   795   800

Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp
            805   810   815

Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr
            820   825   830

Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile
        835   840   845

Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile
    850 .   855   860

Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu
865   870   875   880

Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro
            885   890   895

Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys

                      900   905   910

Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser
        915   920   925

Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala
        930   935   940

Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser
945   950   955   960

Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys
                965   970   975

Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile
              980   985   990

Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val
        995   1000   1005

Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly
        1010   1015   1020

Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
        1025   1030   1035

<210> 21
<211> 1506
<212> DNA
<213> Homo sapiens

<400> 21
cagggcaagg aagtggtact gctggacttt gctgcagctg gaggggagct cggctggctc   60

acacacccgt atggcaaagg gtgggacctg atgcagaaca tcatgaatga catgccgatc   120

tacatgtact ccgtgtgcaa cgtgatgtct ggcgaccagg acaactggct ccgcaccaac   180

tgggtgtacc gaggagaggc tgagcgtatc ttcattgagc tcaagtttac tgtacgtgac   240

tgcaacagct ccctggtgg cgccagctcc tgcaaggaga ctttcaacct ctactatgcc   300

gagtcggacc tggactacgg caccaacttc agaagcgcc tgttcaccaa gattgacacc   360

attgcgcccg atgagatcac cgtcagcagc gacttcgagg cacgccacgt gaagctgaac   420

gtggaggagc gctccgtggg gccgctcacc cgcaaaggct tctacctggc cttccaggat   480

atcggtgcct gtgtggcgct gctctccgtc cgtgtctact acaagaagtg ccccgagctg   540

ctgcagggcc tggcccactt ccctgagacc atcgccggct ctgatgcacc ttccctggcc   600

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actgtggccg gcacctgtgt ggaccatgcc gtggtgccac cggggggtga agagccccgt     660

atgcactgtg cagtggatgg cgagtggctg gtgcccattg ggcagtgcct gtgccaggca     720

ggctacgaga aggtggagga tgcctgccag gcctgctcgc ctggattttt taagtttgag     780

gcatctgaga gcccctgctt ggagtgccct gagcacacgc tgccatcccc tgagggtgcc     840

acctcctgcg agtgtgagga aggcttcttc cgggcacctc aggacccagc gtcgatgcct     900

tgcacgac cccctccgc cccacactac ctcacagccg tgggcatggg tgccaaggtg     960

gagctgcgct ggacgccccc tcaggacagc gggggccgcg aggacattgt ctacagcgtc     1020

acctgcgaac agtgctggcc cgagtctggg gaatgcgggc cgtgtgaggc cagtgtgcgc     1080

tactcggagc ctcctcacgg actgacccgc accagtgtga cagtgagcga cctggagccc     1140

cacatgaact acaccttcac cgtggaggcc cgcaatggcg tctcaggcct ggtaaccagc     1200

cgcagcttcc gtactgccag tgtcagcatc aaccagacag agcccccaa ggtgaggctg     1260

gagggccgca gcaccacctc gcttagcgtc tcctggagca tcccccgcc gcagcagagc     1320

cgagtgtgga agtacgaggt cacttaccgc aagaagggag actccaacag ctacaatgtg     1380

cgccgcaccg agggtttctc cgtgaccctg gacgacctgg ccccagacac cacctacctg     1440

gtccaggtgc aggcactgac gcaggagggc caggggggccg gcagcagggt gcacgaattc     1500

cagacg                                                                1506
```

```
<210>  22
<211>  1506
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Human sequence optimized for
codon usage in Listeria

<400>  22
caaggtaaag aagttgtttt attagatttt gcagcagcag gtggtgaatt aggttggtta     60

acacatccat atggtaaagg ttgggattta atgcaaaata ttatgaatga tatgccaatt     120

tatatgtata gtgtttgtaa tgttatgagt ggtgatcaag ataattggtt acgtacaaat     180

tgggtttatc gtggtgaagc agaacgtatt tttattgaat taaaatttac agttcgtgat     240

tgtaatagtt ttccaggtgg tgcaagtagt tgtaaagaaa catttaattt atattatgca     300

gaaagtgatt tagattatgg tacaaatttt caaaaacgtt tatttacaaa aattgataca     360

attgcaccag atgaaattac agttagtagt gattttgaag cacgtcatgt taaattaaat     420

gttgaagaac gtagtgtttgg tccattaaca cgtaaaggtt tttatttagc atttcaagat     480

attggtgcat gtgttgcatt attaagtgtt cgtgtttatt ataaaaaatg tccagaatta     540
```

```
ttacaaggtt tagcacattt tccagaaaca attgcaggta gtgatgcacc aagtttagca    600

acagttgcag gtacatgtgt tgatcatgca gttgttccac caggtggtga agaaccacgt    660

atgcattgtg cagttgatgg tgaatggtta gttccaattg gtcaatgttt atgtcaagca    720

ggttatgaaa aagttgaaga tgcatgtcaa gcatgtagtc caggtttttt taaatttgaa    780

gcaagtgaaa gtccatgttt agaatgtcca gaacatacat taccaagtcc agaaggtgca    840

acaagttgtg aatgtgaaga aggttttttt cgtgcaccac aagatccagc aagtatgcca    900

tgtacacgtc caccaagtgc accacattat ttaacagcag ttggtatggg tgcaaaagtt    960

gaattacgtt ggacaccacc acaagatagt ggtggtcgtg aagatattgt ttatagtgtt   1020

acatgtgaac aatgttggcc agaaagtggt gaatgtggtc catgtgaagc aagtgttcgt   1080

tatagtgaac caccacatgg tttaacacgt acaagtgtta cagttagtga tttagaacca   1140

catatgaatt atacatttac agttgaagca cgtaatggtg ttagtggttt agttacaagt   1200

cgtagttttc gtacagcaag tgttagtatt aatcaaacag aaccaccaaa agttcgttta   1260


gaaggtcgta gtacaacaag tttaagtgtt agttggagta ttccaccacc acaacaaagt   1320

cgtgtttgga aatatgaagt tacatatcgt aaaaaaggtg atagtaatag ttataatgtt   1380

cgtcgtacag aaggttttag tgttacatta gatgatttag caccagatac aacatattta   1440

gttcaagttc aagcattaac acaagaaggt caaggtgcag gtagtcgtgt tcatgaattt   1500

caaaca                                                              1506
```

```
<210>  23
<211>  502
<212>  PRT
<213>  Homo sapeins
```

```
<400>  23
```

```
Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu
1               5                   10                  15


Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln
            20                  25                  30


Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val
        35                  40                  45


Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg
    50                  55                  60


Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp
```

```
        65                    70                    75                    80


        Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn
                        85                    90                    95


        Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys
                        100                   105                   110


        Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val
                        115                   120                   125


        Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg
            130                   135                   140


        Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp
        145                   150                   155                   160


        Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys
                        165                   170                   175


        Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala
                        180                   185                   190


        Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp
                        195                   200                   205


        His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala
            210                   215                   220


        Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala
        225                   230                   235                   240


        Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe
                        245                   250                   255


        Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His
                        260                   265                   270


        Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly
                        275                   280                   285


        Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro
            290                   295                   300


        Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val
        305                   310                   315                   320
```

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Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile
          325   330   335

Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys
          340   345   350

Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu
          355   360   365

Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr
     370   375   380

Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser
385   390   395   400

Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro
          405   410   415

Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp
          420   425   430

Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr
          435   440   445

Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu
     450   455   460

Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu
465   470   475   480

Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg
          485   490   495

Val His Glu Phe Gln Thr
          500

<210> 24
<211> 1689
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 24
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa   60

```
caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca      120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc      180

gagcagggca aggaagtggt actgctggac tttgctgcag ctggagggga gctcggctgg      240

ctcacacacc cgtatggcaa agggtgggac ctgatgcaga acatcatgaa tgacatgccg      300

atctacatgt actccgtgtg caacgtgatg tctggcgacc aggacaactg gctccgcacc      360

aactgggtgt accgaggaga ggctgagcgt atcttcattg agctcaagtt tactgtacgt      420

gactgcaaca gcttccctgg tggcgccagc tcctgcaagg agactttcaa cctctactat      480

gccgagtcgg acctggacta cggcaccaac ttccagaagc gcctgttcac caagattgac      540

accattgcgc ccgatgagat caccgtcagc agcgacttcg aggcacgcca cgtgaagctg      600

aacgtggagg agcgctccgt ggggccgctc acccgcaaag gcttctacct ggccttccag      660

gatatcggtg cctgtgtggc gctgctctcc gtccgtgtct actacaagaa gtgccccgag      720

ctgctgcagg gcctggccca cttccctgag accatcgccg gctctgatgc accttccctg      780

gccactgtgg ccggcacctg tgtggaccat gccgtggtgc caccgggggg tgaagagccc      840

cgtatgcact gtgcagtgga tggcgagtgg ctggtgccca ttgggcagtg cctgtgccag      900

gcaggctacg agaaggtgga ggatgcctgc aggcctgct cgcctggatt ttttaagttt      960

gaggcatctg agagccctg cttggagtgc cctgagcaca cgctgccatc ccctgagggt     1020

gccacctcct gcgagtgtga ggaaggcttc ttccgggcac ctcaggaccc agcgtcgatg     1080

ccttgcacac gaccccctc cgccccacac tacctcacag ccgtgggcat gggtgccaag     1140

gtggagctgc gctggacgcc ccctcaggac agcggggggcc gcgaggacat tgtctacagc     1200

gtcacctgcg aacagtgctg gcccgagtct ggggaatgcg ggccgtgtga ggccagtgtg     1260

cgctactcgg agcctcctca cggactgacc cgcaccagtg tgacagtgag cgacctggag     1320

ccccacatga actacacctt caccgtggag gcccgcaatg gcgtctcagg cctggtaacc     1380

agccgcagct tccgtactgc cagtgtcagc atcaaccaga cagagccccc caaggtgagg     1440

ctggagggcc gcagcaccac ctcgcttagc gtctcctgga gcatcccccc gccgcagcag     1500

agccgagtgt ggaagtacga ggtcacttac cgcaagaagg gagactccaa cagctacaat     1560

gtgcgccgca ccgagggttt ctccgtgacc ctggacgacc tggccccaga caccacctac     1620

ctggtccagg tgcaggcact gacgcaggag ggccaggggg ccggcagcag ggtgcacgaa     1680

ttccagacg                                                            1689
```

```
<210>   25
<211>   563
<212>   PRT
<213>   Artificial Sequence
```

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 25

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1   5   10   15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
                20   25   30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
            35   40   45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Gln Gly Lys
        50   55   60

Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp
65   70   75   80

Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met
                85   90   95

Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
            100   105   110

Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala
        115   120   125

Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser
    130   135   140

Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr
145   150   155   160

Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe
                165   170   175

Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp
            180   185   190

Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly
        195   200   205

Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala
    210   215   220

Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu
225   230   235   240

Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp
              245   250   255

Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala Val
              260   265   270

Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
              275   280   285

Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
    290   295   300

Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
305   310   315   320

Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro
              325   330   335

Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
              340   345   350

Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala
              355   360   365

Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg
    370   375   380

Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
385   390   395   400

Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
              405   410   415

Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
              420   425   430

Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
              435   440   445

Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe
450   455   460

Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
465   470   475   480

Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
                485   490   495
                                                            .

Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys
            500   505   510

Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser
            515   520   525

Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
        530   535   540

Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His Glu
545   550   555   560

Phe Gln Thr

<210>   26
<211>   1989
<212>   DNA
<213>   Artificial Sequence

<220>
<223>   Description of Artificial Sequence: Fusion protein construct

<400>   26
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac   60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata   120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg   180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg   240

aaaaaaataa tgctagtttt tattcacactt atattagtta gtctaccaat tgcgcaacaa   300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca   360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc   420

gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca   480

gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa   540

aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat   600

caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tatttttatt   660

```
gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa      720

gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa      780

cgtttattta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt      840

gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa      900

ggttttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt      960

tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca     1020

ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt     1080

ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca     1140

attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt     1200

agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat     1260

acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca     1320

ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca     1380

gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt     1440

cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt     1500

ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt     1560

gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat     1620

ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa     1680

acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg     1740

agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa     1800

ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat     1860

ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt     1920

gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta     1980

tgagagctc                                                             1989
```

<210> 27
<211> 581
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 27

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1   5   10   15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
        20   25   30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35   40   45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
        50   55   60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65   70   75   80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
                85   90   95

Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
            100   105   110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
        115   120   125

Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
    130   135   140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145   150   155   160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
            165   170   175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
            180   185   190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
        195   200   205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
    210   215   220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225   230   235   240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
            245   250   255

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Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
        260   265   270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
        275   280   285

Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
    290   295   300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305   310   315   320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
            325   330   335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
        340   345   350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
        355   360   365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
    370   375   380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly
385   390   395   400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
            405   410   415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
        420   425   430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
        435   440   445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
    450   455   460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465   470   475   480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
            485   490   495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp

                    500   505   510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
        515   520   525

Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
    530   535   540

Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545   550   555   560

Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
                565   570   575

Ser Glu Glu Asp Leu
            580

<210> 28
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Construct for fusion protein

<400> 28
ggtacctcct tgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac   60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata   120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg   180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg   240

aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa   300

acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca   360

ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaacatgc agatggatcc   420

gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca   480

gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa   540

aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat   600

caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tattttatt   660

gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa   720

gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa   780

cgtttatta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt   840

gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa   900

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```
ggtttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt      960

tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca     1020

ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt     1080

ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca     1140

attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt     1200

agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat     1260

acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca     1320

ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca     1380

gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt     1440

cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt     1500

ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt     1560

gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat     1620

ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa     1680

acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg     1740

agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa     1800

ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat     1860

ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt     1920

gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta     1980

tgagagctc                                                            1989
```

<210>   29
<211>   581
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Description of Artificial Sequence: Predicted Fusion protein

<400>   29

```
Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15


Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30


Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45
```

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
    50   55   His   60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65   70   Glu   75   80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
            85   90   95

Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
            100   105   110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
        115   120   125

Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
    130   135   140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145   150   155   160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
            165   170   175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
            180   185   190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
        195   200   205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
    210   215   220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225   230   235   240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
            245   250   255

Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
            260   265   270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
            275   280   285

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Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
    290   295   300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305   310   315   320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
                325   330   335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
                340   345   350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
                355   360   365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
    370   375   380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly

385   390   395   400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
                405   410   415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
                420   425   430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
                435   440   445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
    450   455   460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465   470   475   480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
                485   490   495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp
                500   505   510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
    515   520   525

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```
Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
    530               535           540
```

```
Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545               550               555               560
```

```
Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
                565           570               575
```

```
Ser Glu Glu Asp Leu
            580
```

```
<210>  30
<211>  1968
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Fusion protein construct

<400>  30
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac    60
atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata   120
atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg   180
gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg   240
gcatacgaca gtcgttttga tgaatgggta cagaaactga agaggaaag ctttcaaaac    300
aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt   360
ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat   420
aaacaaggta agaagttgt tttattagat tttgcagcag caggtggtga attaggttgg    480
ttaacacatc catatggtaa aggttgggat ttaatgcaaa atattatgaa tgatatgcca   540
atttatatgt atagtgtttg taatgttatg agtggtgatc aagataattg gttacgtaca   600
aattgggttt atcgtggtga agcagaacgt attttttattg aattaaaatt tacagttcgt   660
gattgtaata gttttccagg tggtgcaagt agttgtaaag aaacatttaa tttatattat   720
gcagaaagtg atttagatta tggtacaaat tttcaaaaac gtttatttac aaaaattgat   780
acaattgcac cagatgaaat tacagttagt agtgattttg aagcacgtca tgttaaatta   840
aatgttgaag aacgtagtgt tggtccatta acacgtaaag gttttattt agcatttcaa    900
gatattggtg catgtgttgc attattaagt gttcgtgttt attataaaaa atgtccagaa   960
ttattacaag gtttagcaca ttttccagaa acaattgcag gtagtgatgc accaagttta  1020
gcaacagttg caggtacatg tgttgatcat gcagttgttc caccaggtgg tgaagaacca  1080
```

```
cgtatgcatt gtgcagttga tggtgaatgg ttagttccaa ttggtcaatg tttatgtcaa    1140

gcaggttatg aaaaagttga agatgcatgt caagcatgta gtccaggttt ttttaaattt    1200

gaagcaagtg aaagtccatg tttagaatgt ccagaacata cattaccaag tccagaaggt    1260

gcaacaagtt gtgaatgtga agaaggtttt tttcgtgcac cacaagatcc agcaagtatg    1320

ccatgtacac gtccaccaag tgcaccacat tatttaacag cagttggtat gggtgcaaaa    1380

gttgaattac gttggacacc accacaagat agtggtggtc gtgaagatat tgtttatagt    1440

gttacatgtg aacaatgttg gccagaaagt ggtgaatgtg gtccatgtga agcaagtgtt    1500

cgttatagtg aaccaccaca tggtttaaca cgtacaagtg ttacagttag tgatttagaa    1560

ccacatatga attatacatt tacagttgaa gcacgtaatg gtgttagtgg tttagttaca    1620

agtcgtagtt ttcgtacagc aagtgttagt attaatcaaa cagaaccacc aaaagttcgt    1680

ttagaaggtc gtagtacaac aagtttaagt gttagttgga gtattccacc accacaacaa    1740

agtcgtgttt ggaaatatga agttacatat cgtaaaaaag gtgatagtaa tagttataat    1800

gttcgtcgta cagaaggttt tagtgttaca ttagatgatt tagcaccaga tacaacatat    1860

ttagttcaag ttcaagcatt aacacaagaa ggtcaaggtg caggtagtcg tgttcatgaa    1920

tttcaaacag aacaaaaatt aattagtgaa gaagatttat gagagctc                 1968
```

```
<210> 31
<211> 574
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Predicted Fusion Protein

<400> 31

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1               5                   10                  15


Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
            20                  25                  30


Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
        35                  40                  45


Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys Gln Gly
    50                  55                  60


Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly
65                  70                  75                  80
```

```
Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile
            85                  90                  95


Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser
            100             105                 110


Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu
            115             120                 125


Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn
            130             135                 140


Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr
145             150             155                 160


Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu
            165             170                 175


Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser
            180             185                 190


Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val
            195             200                 205


Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly
210             215                 220


Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro
225             230             235                 240


Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser
            245             250                 255


Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala
            260             265                 270


Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp
            275             280                 285


Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr
            290             295                 300


Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys
305             310             315                 320
```

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Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu
            325   330   335

Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe
            340   345   350

Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser
            355   360   365

Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu
    370   375   380

Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr
385   390   395   400

Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro
            405   410   415

Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg
            420   425   430

Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe
            435   440   445

Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser
    450   455   460

Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val
465   470   475   480

Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile
            485   490   495

Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg
            500   505   510

Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe
            515   520   525

Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln
    530   535   540

Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His
545   550   555   560

Glu Phe Gln Thr Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu

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565   570

<210> 32
<211> 1254
<212> DNA
<213> Homo sapiens

<400> 32
caccgcagga ggaagaacca gcgtgcccgc cagtccccgg aggacgttta cttctccaag   60

tcagaacaac tgaagcccct gaagacatac gtggacccccc acacatatga ggacccccaac   120

caggctgtgt tgaagttcac taccgagatc catccatcct gtgtcactcg gcagaaggtg   180

atcggagcag gagagtttgg ggaggtgtac aagggcatgc tgaagacatc ctcggggaag   240

aaggaggtgc cggtggccat caagacgctg aaagccggct acacagagaa gcagcgagtg   300

gacttcctcg gcgaggccgg catcatgggc cagttcagcc accacaacat catccgccta   360

gagggcgtca tctccaaata caagcccatg atgatcatca ctgagtacat ggagaatggg   420

gccctggaca agttccttcg ggagaaggat ggcgagttca gcgtgctgca gctggtgggc   480

atgctgcggg gcatcgcagc tggcatgaag tacctggcca acatgaacta tgtgcaccgt   540

gacctggctg cccgcaacat cctcgtcaac agcaacctgg tctgcaaggt gtctgacttt   600

ggcctgtccc gcgtgctgga ggacgacccc gaggccacct acaccaccag tggcggcaag   660

atccccatcc gctggaccgc cccggaggcc atttcctacc ggaagttcac ctctgccagc   720

gacgtgtgga gctttggcat tgtcatgtgg gaggtgatga cctatggcga gcggccctac   780

tgggagttgt ccaaccacga ggtgatgaaa gccatcaatg atggcttccg gctccccaca   840

cccatggact gcccctccgc catctaccag ctcatgatgc agtgctggca gcaggagcgt   900

gcccgccgcc ccaagttcgc tgacatcgtc agcatcctgg acaagctcat tcgtgcccct   960

gactccctca agaccctggc tgactttgac ccccgcgtgt ctatccggct ccccagcacg   1020

agcggctcgg aggggggtgcc cttccgcacg gtgtccgagt ggctggagtc catcaagatg   1080

cagcagtata cggagcactt catggcggcc ggctacactg ccatcgagaa ggtggtgcag   1140

atgaccaacg acgacatcaa gaggattggg gtgcggctgc ccggccacca gaagcgcatc   1200

gcctacagcc tgctgggact caaggaccag gtgaacactg tggggatccc catc   1254

<210> 33
<211> 1254
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sequence Optimized for codon
usage in Listeria

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<400> 33
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cacagacgta gaaaaaatca acgtgctcga caatccccag aagatgtgta tttttcgaaa      60

agtgaacaat taaaaccatt aaaaacttat gttgatccgc atacgtacga agacccaaat     120

caagcagtat taaaatttac aacagaaata cacccaagtt gtgttacaag acaaaaagtt     180

attggagcag gtgaattcgg agaggtatat aaaggtatgt taaaaacatc atcaggtaaa     240

aaagaagttc cggttgcaat taaaacctta aaggcaggat atacagaaaa acagcgagtt     300

gattttttag gtgaagcagg aattatgggt caatttagcc atcataatat tattcgtttg     360

gaaggagtaa taagtaaata taaaccaatg atgattatta cagaatacat ggaaaacggt     420

gctttagata aattttttacg tgaaaaggat ggtgaattta gtgttttaca attggttggt     480

atgttaagag gaattgctgc aggtatgaaa tatttagcta atatgaatta tgttcaccgt     540

gatttggcag caagaaatat cctagtcaat tccaatttag tatgtaaagt tagtgatttt     600

ggtttaagca gagtattaga agacgatcca gaggcaacct atacaacatc gggaggtaaa     660

attcctattc gttggacagc accagaagct atcagttacc gtaaatttac aagtgcatca     720

gacgtgtgga gttttgggat tgtaatgtgg gaagttatga catatggaga aagaccatat     780

tgggaattaa gtaatcatga agttatgaaa gcaattaacg atggatttag attaccaact     840

ccgatggatt gtccatctgc catttatcaa ctaatgatgc aatgttggca caagaaaga      900

gcacgacgtc caaaatttgc agatattgtt agtattttag acaaattaat tcgtgcacca     960

gatagtttaa aaactttagc agactttgat cctcgtgtta gtattcgatt accaagtacg    1020

tcaggttccg aaggagttcc atttcgcaca gtctccgaat ggttggaatc aattaaaatg    1080

caacaataca ccgaacactt tatggcagca ggttacacag caatcgaaaa agttgttcaa    1140

atgacaaatg atgatattaa acgtattgga gttagattac caggccacca gaaacgtatt    1200

gcatattctt tattaggttt aaaagatcaa gttaataccg tgggaattcc aatt          1254
```

<210> 34
<211> 456
<212> PRT
<213> Homo sapiens

<400> 34

```
Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala
1               5                   10                  15


Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala
            20                  25                  30


Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln Arg Ala Arg
        35                  40                  45
```

Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro
    50   55   60

Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala
65   70   75   80

Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln
            85   90   95

Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu
            100   105   110

Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu
            115   120   125

Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala
    130   135   140

Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly
145   150   155   160

Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu
            165   170   175

Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser
            180   185   190

Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys
    195   200   205

Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn
    210   215   220

Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu
225   230   235   240

Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly
            245   250   255

Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg
            260   265   270

Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp
    275   280   285

Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His
    290   295   300

Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met
305   310   315   320

Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln
            325   330   335

Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp
        340   345   350

Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp
        355   360   365

Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val
    370   375   380

Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln
385   390   395   400

Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val
            405   410   415

Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro
        420   425   430

Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln
        435   440   445

Val Asn Thr Val Gly Ile Pro Ile
    450   455

<210> 35
<211> 1437
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion Protein

<400> 35
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa   60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca   120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc   180

```
gagcaccgca ggaggaagaa ccagcgtgcc cgccagtccc cggaggacgt ttacttctcc      240

aagtcagaac aactgaagcc cctgaagaca tacgtggacc cccacacata tgaggacccc      300

aaccaggctg tgttgaagtt cactaccgag atccatccat cctgtgtcac tcggcagaag      360

gtgatcggag caggagagtt tggggaggtg tacaagggca tgctgaagac atcctcgggg      420

aagaaggagg tgccggtggc catcaagacg ctgaaagccg gctacacaga gaagcagcga      480

gtggacttcc tcggcgaggc cggcatcatg ggccagttca gccaccacaa catcatccgc      540

ctagagggcg tcatctccaa atacaagccc atgatgatca tcactgagta catggagaat      600

ggggccctgg acaagttcct tcgggagaag gatggcgagt tcagcgtgct gcagctggtg      660

ggcatgctgc ggggcatcgc agctggcatg aagtacctgg ccaacatgaa ctatgtgcac      720

cgtgacctgg ctgcccgcaa catcctcgtc aacagcaacc tggtctgcaa ggtgtctgac      780

tttggcctgt cccgcgtgct ggaggacgac cccgaggcca cctacaccac cagtggcggc      840


aagatcccca tccgctggac cgccccggag gccatttcct accggaagtt cacctctgcc      900

agcgacgtgt ggagctttgg cattgtcatg tgggaggtga tgacctatgg cgagcggccc      960

tactgggagt tgtccaacca cgaggtgatg aaagccatca atgatggctt ccggctcccc     1020

acacccatgg actgcccctc cgccatctac cagctcatga tgcagtgctg cagcaggag     1080

cgtgcccgcc gccccaagtt cgctgacatc gtcagcatcc tggacaagct cattcgtgcc     1140

cctgactccc tcaagaccct ggctgacttt gaccccgcg tgtctatccg gctccccagc     1200

acgagcggct cggagggggt gcccttccgc acggtgtccg agtggctgga gtccatcaag     1260

atgcagcagt atacggagca cttcatggcg gccggctaca ctgccatcga gaaggtggtg     1320

cagatgacca acgacgacat caagaggatt ggggtgcggc tgcccggcca ccagaagcgc     1380

atcgcctaca gcctgctggg actcaaggac caggtgaaca ctgtggggat ccccatc       1437
```

```
<210>  36
<211>  479
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Predicted Protein Sequence

<400>  36

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15


Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30
```

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
         35   40   45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu His Arg Arg
     50   55   60

Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser
65   70   75   80

Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr
             85   90   95

Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His
         100   105   110

Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly
         115   120   125

Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val
     130   135   140

Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg
145   150   155   160

Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His
             165   170   175

Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met
             180   185   190

Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg
         195   200   205

Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg
     210   215   220

Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His
225   230   235   240

Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys
             245   250   255

Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu
             260   265   270

Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala
        275   280   285

Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp
    290   295   300

Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro
305   310   315   320

Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly
                325   330   335

Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu
            340   345   350

Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala
        355   360   365

Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu
    370   375   380

Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser
385   390   395   400

Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu
            405   410   415

Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly
        420   425   430

Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile Lys
        435   440   445

Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr Ser
    450   455   460

Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
465   470   475

<210> 37
<211> 1737
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein sequence

<400> 37

-45-

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ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac      60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata     120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg     180


gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg     240

aaaaaaataa tgctagtttt tattacactt atattagtta gtctaccaat tgcgcaacaa     300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca     360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc     420

gattataaag atgatgatga taaacacaga cgtagaaaaa tcaacgtgc tcgacaatcc     480

ccagaagatg tgtatttttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat     540

ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca     600

agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt     660

atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca     720

ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt     780

agccatcata atattattcg tttggaagga gtaataagta aatataaacc aatgatgatt     840

attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa     900

tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta     960

gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat    1020

ttagtatgta aagttagtga tttttggttta agcagagtat tagaagacga tccagaggca    1080

acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt    1140

taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt    1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt    1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg    1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt    1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt    1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc    1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac    1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga    1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat    1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata gagctc      1737
```

<210> 38

```
<211>   497
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Description of Artificial Sequence:  Predicted fusion protein

<400>   38

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15


Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30


Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45


Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
        50                  55                  60


Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln
65                  70                  75                  80


Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
                85                  90                  95


Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
                100                 105                 110


Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
            115                 120                 125


Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
        130                 135                 140


Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145                 150                 155                 160


Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
                165                 170                 175


Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
            180                 185                 190


Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
            195                 200                 205
```

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
    210   215   220

Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225   230   235   240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
            245   250   255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
            260   265   270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
        275   280   285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
    290   295   300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305   310   315   320

Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
            325   330   335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp
            340   345   350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
        355   360   365

Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
    370   375   380

Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385   390   395   400

Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
            405   410   415

Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
            420   425   430

Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
        435   440   445

Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly

                450   455   460

        His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
        465   470   475   480

        Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
                        485   490   495

        Leu

        <210> 39
        <211> 1737
        <212> DNA
        <213> Artificial Sequence

        <220>
        <223> Description of Artificial Sequence: Fusion protein construct

        <400> 39
        ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac   60

        atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata   120

        atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg   180

        gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg   240

        aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa   300

        acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca   360

        ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaacatgc agatggatcc   420

        gattataaag acgatgatga taaacacaga cgtagaaaaa atcaacgtgc tcgacaatcc   480

        ccagaagatg tgtattttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat   540

        ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca   600

        agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt   660

        atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca   720

        ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt   780

        agccatcata tattattcg tttggaagga gtaataagta aatataaacc aatgatgatt   840

        attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa   900

        tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta   960

        gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat   1020

        ttagtatgta aagttagtga ttttggttta agcagagtat tagaagacga tccagaggca   1080

        acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt   1140

```
taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt    1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt    1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg    1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt    1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt    1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc    1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac    1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga    1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat    1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata agagctc       1737
```

```
<210>   40
<211>   497
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Description of Artificial Sequence:  Predicted Fusion Protein

<400>   40
```

```
Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15


Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30


Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45


Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
    50                  55                  60


Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln
65                  70                  75                  80


Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
                85                  90                  95


Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
            100                 105                 110


Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
```

                    115   120   125

Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
    130   135   140

Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145   150   .   155   160

Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
                165   170   175

Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
                180   185   190

Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
                195   200   205

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
    210   215   220

Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225   230   235   240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
                245   250   255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
                260   265   270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
    275   280   285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
    290   295   300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305   310   315   320

Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
                325   330   335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp
                340   345   350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
                355   360   365

                                    -51-

```
Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
    370             375             380
```

```
Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385             390             395             400
```

```
Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
                405             410             415
```

```
Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
            420             425             430
```

```
Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
        435             440             445
```

```
Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly
    450             455             460
```

```
His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
465             470             475             480
```

```
Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
                485             490             495
```

```
Leu
```

```
<210>  41
<211>  1716
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Fusion protein construct

<400>  41
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac      60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata     120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg     180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg     240

gcatacgaca gtcgttttga tgaatgggta cagaaactga aagaggaaag ctttcaaaac     300

aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt     360

ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat     420
```

```
aaacacagac gtagaaaaaa tcaacgtgct cgacaatccc cagaagatgt gtatttttcg    480

aaaagtgaac aattaaaacc attaaaaact tatgttgatc cgcatacgta cgaagaccca    540

aatcaagcag tattaaaatt tacaacagaa atacacccaa gttgtgttac aagacaaaaa    600

gttattggag caggtgaatt cggagaggta tataaaggta tgttaaaaac atcatcaggt    660

aaaaaagaag ttccggttgc aattaaaacc ttaaaggcag gatatacaga aaaacagcga    720

gttgattttt taggtgaagc aggaattatg ggtcaattta gccatcataa tattattcgt    780

ttggaaggag taataagtaa atataaacca atgatgatta ttacagaata catggaaaac    840

ggtgctttag ataaattttt acgtgaaaag gatggtgaat ttagtgtttt acaattggtt    900

ggtatgttaa gaggaattgc tgcaggtatg aaatatttag ctaatatgaa ttatgttcac    960

cgtgatttgg cagcaagaaa tatcctagtc aattccaatt tagtatgtaa agttagtgat   1020

tttggtttaa gcagagtatt agaagacgat ccagaggcaa cctatacaac atcgggaggt   1080

aaaattccta ttcgttggac agcaccagaa gctatcagtt accgtaaatt tacaagtgca   1140

tcagacgtgt ggagttttgg gattgtaatg tgggaagtta tgacatatgg agaaagacca   1200

tattgggaat taagtaatca tgaagttatg aaagcaatta cgatggatt tagattacca    1260

actccgatgg attgtccatc tgccatttat caactaatga tgcaatgttg gcaacaagaa   1320

agagcacgac gtccaaaatt tgcagatatt gttagtattt tagacaaatt aattcgtgca   1380

ccagatagtt taaaaacttt agcagacttt gatcctcgtg ttagtattcg attaccaagt   1440

acgtcaggtt ccgaaggagt tccatttcgc acagtctccg aatggttgga atcaattaaa   1500

atgcaacaat acaccgaaca ctttatggca gcaggttaca cagcaatcga aaaagttgtt   1560

caaatgacaa atgatgatat taaacgtatt ggagttagat taccaggcca ccagaaacgt   1620

attgcatatt ctttattagg tttaaaagat caagttaata ccgtgggaat tccaattgaa   1680

caaaaattaa tttccgaaga agacttataa gagctc                             1716
```

<210> 42
<211> 490
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 42

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1   5   10   15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly

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                    20   25   30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
        35   40   45

Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Lys His Arg
        50   55   60

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
65   70   75   80

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
                85   90   95

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
            100   105   110

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
        115   120   125

Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
    130   135   140

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
145   150   155   160

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
            165   170   175

His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
            180   185   190

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
        195   200   205

Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
    210   215   220

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
225   230   235   240

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
            245   250   255

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
            260   265   270

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
        275   280   285

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
    290   295   300

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
305   310   315   320

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
                325   330   335

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
        340   345   350

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
        355   360   365

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
    370   375   380

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
385   390   395   400

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
                405   410   415

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
                420   425   430

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
        435   440   445

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
    450   455   460

Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
465   470   475   480

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
                485   490

<210> 43
<211> 9808
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion Protein Construct

<400> 43

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ctttaaacgt ggatcatttt ctttaaattt atgctgacga cctttgaatt tgcctttttt      60

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ttgatacttg tacaattcaa tgacaaggct attaatcaaa cgccttaaat tttcatcttc     180

aataccattc attgagggta aatttaagac ttccagggtt gcccccttaa tttgaatttg     240

attcatcaat tctgttaatt ctttattatt tcgtcctaat cgatctaatt cagtaacaat     300

aacaatatcc ccttcacgaa tatagttaag catagcttgt aattgtgggc gttcgaccga     360

ttgaccgctt aatttgtctg aaaagacctt agaaacgccc tgtaacgctt gtaattgccg     420

atctaagttc tgttctttgc tactgacacg tgcataacca attttagcca ttttcaacca     480

acctctaaaa ttctctcggt tgcaataacc aatcagcaat atctactttt tcaatttcaa     540

attgcttatc agaaattgtc ttttcgtaag cgataaaatc ttgcgcatat tgttgctcat     600

taaaaatagc caccacttcg tcattttcta aaactcgata aataaatttt ttcattttac     660

tcctcctatt atgcccaact aaatgacct attcaccaag tcaattatac tgctaaaatc      720

atattaggac aaataggtat actctattga cctataaatg atagcaactt aaaagatcaa     780

gtgttcgctt cgctctcact gcccctcgac gttttagtag cctttccctc acttcgttca     840

gtccaagcca actaaaagtt ttcgggctac tctctccttc tcccccctaat aattaattaa     900

aatcttactc tgtatatttc tgctaatcat tcactaaaca gcaaagaaaa acaaacacgt     960

atcatagata taaatgtaat ggcatagtgc gggtttttatt ttcagcctgt atcgtagcta    1020

aacaaatcga gttgtgggtc cgttttgggg cgttctgcca atttgtttag agtttcttga    1080

ataaatgtac gttctaaatt aaacgaagct gtcagcgcct ttatatagct ttctcgttct    1140

tctttttttta atttaatgat cgatagcaac aatgatttaa cactagcaag ttgaatgcca    1200

ccatttcttc ctggtttaat cttaaagaaa atttcctgat tcgccttcag taccttcagc    1260

aatttatcta atgtccgttc aggaatgcct agcacttctc taatctcttt tttggtcgtc    1320

gctaaataag gcttgtatac atcgcttttt tcgctaatat aagccattaa atcttctttc    1380

cattctgaca aatgaacacg ttgacgttcg cttctttttt tcttgaattt aaaccaccct    1440

tgacggacaa ataaatcttt actggttaaa tcacttgata cccaagcttt gcaaagaatg    1500

gtaatgtatt ccctattagc cccttgatag ttttctgaat aggcacttct aacaattttg    1560

attacttctt tttcttctaa gggttgatct aatcgattat taaactcaaa catattatat    1620

tcgcacgttt cgattgaata gcctgaacta aagtaggcta aagagagggt aaacataacg    1680
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ctattgcgcc ctactaaacc cttttctcct gaaaatttcg tttcgtgcaa taagagatta   1740

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<213> Listeria monocytogenes

<400> 44

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```

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<210> 45
<211> 59
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<213> Listeria monocytogenes

<400> 45

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1               5                   10                  15
```

```
Glu Asn Lys Gly Met Thr Arg Arg Glu Met Leu Lys Leu Ser Ala Val
            20                  25                  30
```

```
Ala Gly Thr Gly Ile Ala Val Gly Ala Thr Gly Leu Gly Thr Ile Leu
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Asn Val Val Asp Gln Val Asp Lys Ala Leu Thr
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<210> 46
<211> 53
<212> PRT
<213> Bacillus subtillus

<400> 46

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1   5   10   15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
            20   25   30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
        35   40   45

Val Gly Ala Phe Gly
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<220>
<223> Description of Artificial Sequence: Primer

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<210> 48
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<220>
<223> Description of Artificial Sequence: Primer

<400> 48
acataatcag tccaaagtag atgc   24

<210> 49
<211> 29
<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

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<210>  50
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<220>
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<400>  50
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<210>  51
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<220>
<223>  Description of Artificial Sequence:  Epitope insert

<400>  51
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<210>  52
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<220>
<223>  Description of Artificial Sequence:  Epitope

<400>  52

Asp Tyr Lys Asp Asp Asp Asp Lys
1               5


<210>  53
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<220>
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<400>  53
gaacaaaaat taattagtga agaagattta                                   30


<210>  54
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<220>
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<223> Description of Artificial Sequence: Epitope

<400> 54

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1   5   10

<210> 55
<211> 9
<212> PRT
<213> Mus sp.

<400> 55

Ser Pro Ser Tyr Val Tyr His Gln Phe
1   5

<210> 56
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Epitope

<400> 56

Ser Pro Ser Tyr Ala Tyr His Gln Phe
1   5

<210> 57
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<212> DNA
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<220>
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<210> 58
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<220>
<223> Description of Artificial Sequence: Primer

<400> 58
caatggatcc ctcgagatca taatttactt catccc   36

<210> 59
<211> 32
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 59
atttctcgag tccatggggg gttctcatca tc   32

<210> 60
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<220>
<223> Description of Artificial Sequence: Primer

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<210> 61
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<223> Description of Artificial Sequence: Primer

<400> 61
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<210> 62
<211> 31
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<223> Description of Artificial Sequence: Primer

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<210> 63
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Epitope insert

<400> 63

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<210> 64
<211> 33
<212> DNA

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<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Primer

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<211>  34
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Primer

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<210>  66
<211>  8
<212>  RNA
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Consensus Sequence


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<223>  n is a, c, g, or u

<400>  66
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<210>  67
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<212>  DNA
<213>  Listeria monocytogenes

<400>  67
aaggagagtg aaacccatg                                             19


<210>  68
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<212>  DNA
<213>  Listeria monocytogenes

<400>  68
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<213>   Listeria monocytogenes

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<210>   70
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<212>   PRT
<213>   Listeria monocytogenes

<400>   70

Thr Glu Ala Lys Asp
1               5


<210>   71
<211>   5
<212>   PRT
<213>   Listeria monocytogenes

<400>   71

Asp Lys Ala Leu Thr
1               5


<210>   72
<211>   5
<212>   PRT
<213>   Bacillus subtillus

<400>   72

Val Gly Ala Phe Gly
1               5
```